ALUMINIUM PHOSPHIDE NEUROTOXICITY IN ALBINO RAT

ABSTRACT

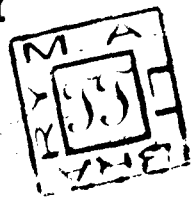
THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY



BY

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DEPARTMENT OF CHEMISTRY
AND
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JAWAHARLAL NEHRU MEDICAL COLLEGE
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Brain is the most susceptible and vulnerable organ of the body, and hence attacking the nervous system is the easiest and surest way of chemically upsetting the body metabolism. It is also one of the most lipid rich regions of the body. Lipid peroxidation is an important lipid-chain degradation process. Nucleic acids and proteins are also important constituents of brain. Sulfhydryl groups and enzymes glutathione-s-transferase (GST) and superoxide dismutase (SOD) are related to the detoxification process against the toxicants.

Considering this great need, we conducted study on AIP poisoning in different regions of brain. In cholinergic system, OFB, lipid metabolism, lipid peroxidation, sulfhydryl groups, SOD, cellular components (DNA, RNA & Protein) of albino rat brain Also protective effects of antioxidant, vitamin E (a-tocopherol) on the adverse effects of AIP, were studied.

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1. AIP (10 mg/kg.b.wt.,2ml/kg. b.wt for 7 days), given by gavage was found to produce the undermentioned observations.

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Maximum depletion occurred in the cerebellum.

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h. Glutathione reductase (GR) activity decreased in all the regions of brain and spinal cord. Maximum reduction is noticed in cerebellum.

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k. The MAO activity increased in vrious regions of the brain and spinal cord. Brain stem showed maximum increment while minimum increase was observed in he spinal cord.

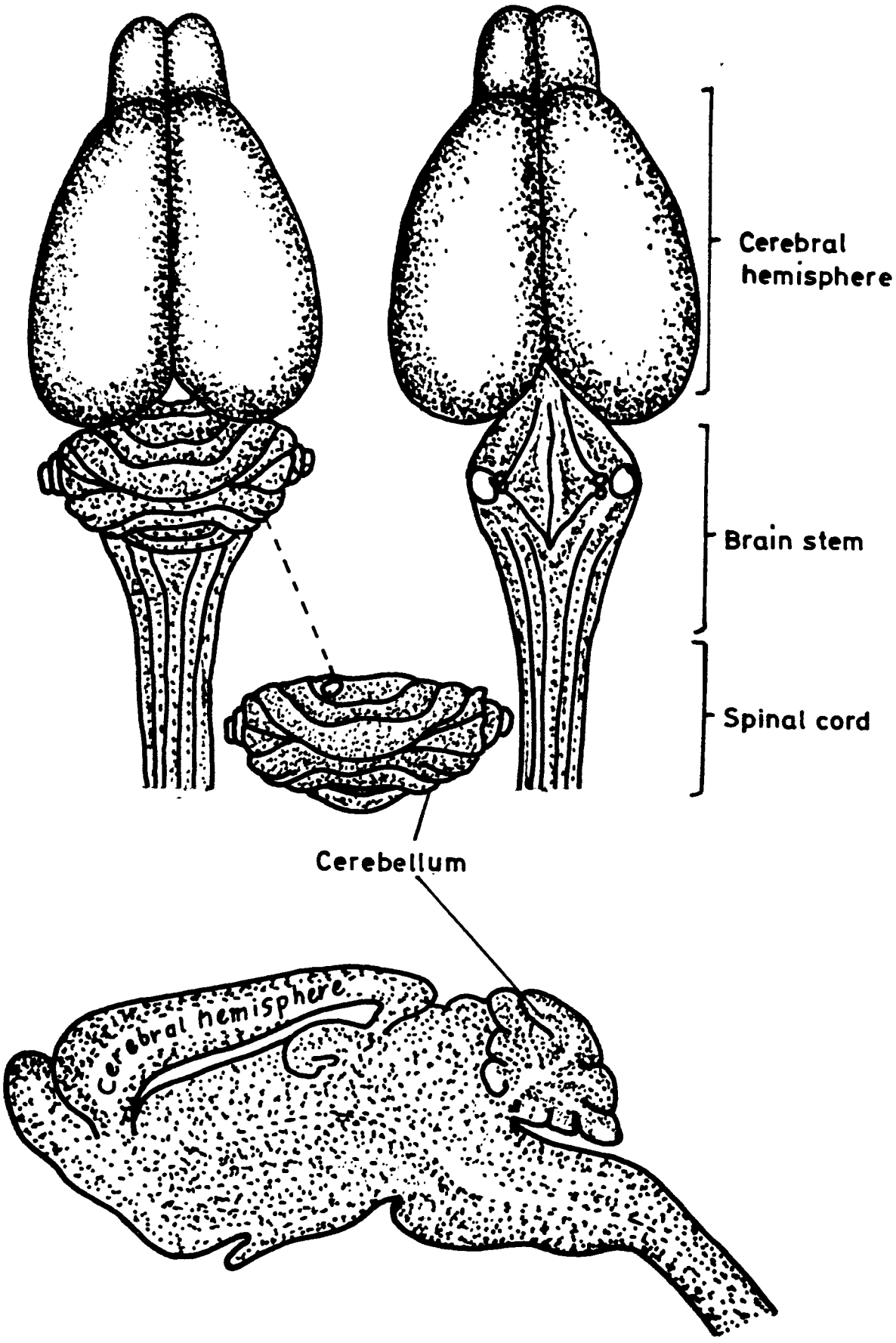
l. The level of DNA concentration was found to decrease significantly in different regions the CNS. Maximum depletion was observed in the cerebellum.

m. The concentration of RNA in all the regions of brain and spinal cord increased with the maxium increment discernible in the cerebellum.

n. The levels of protein were found depleted in different regions of the CNS. Most significant diminution of proteins was observed in the cerebrum.

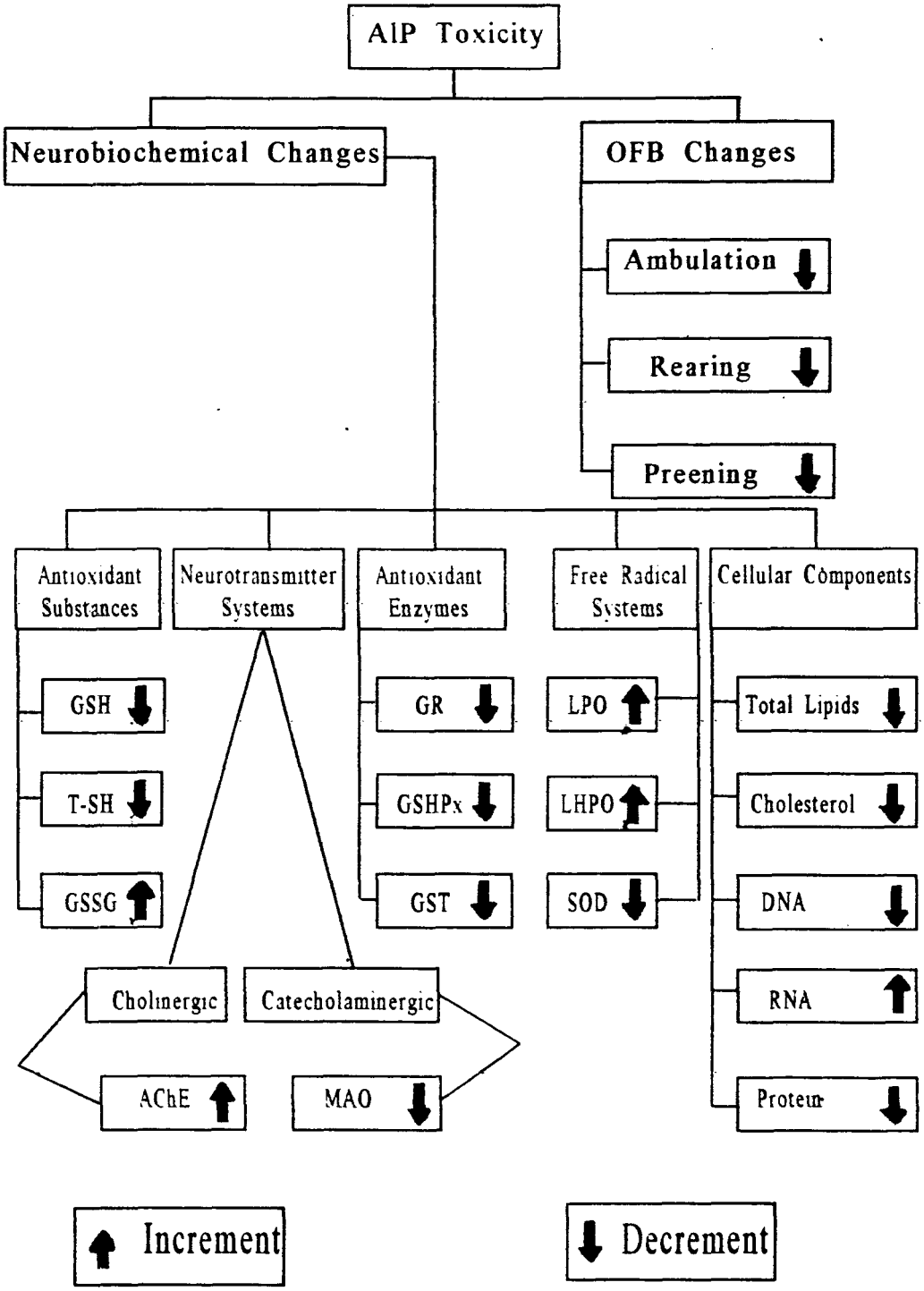
o. The AChE activity was inhibited in various parts of the brain and spinal cord with the maximum depletionwas observed in the spinal cord.

2. Remarkable protection by α-Tocopherol (Vitamin E) administered (150 IU/Kg. b.wt., orally) simulataneously with AIP solution was observed against AIP-induced adverse effects on OFB, LPO, LHPO, GSH, GSSG, GPx, SOD, GST & MAO activities in differetn regions of the brain and spinal cord of rat.



Dissection of different parts of the rat CNS (McEwen and Pfaff 1970.).

Findings of Work in Brief



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ALIGARH (INDIA)

1997

Dedicated
TO
MY
Grand Mother
&
Parents

Phone: Off.: 0571-400515
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Certificate

This is to certify that the research work embodied in this thesis entitled ***"ALUMINIUM PHOSPHIDE NEUROTOXICITY IN ALBINO RAT"*** is the result of the original research work of ***Mr. KUNWAR ASIF*** carried out under my supervision and is suitable for submission for the award of the ***Degree of Doctor of Philosophy in Chemistry*** of this university.

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Certificate

This is to certify that the research work embodied in this thesis entitled *" ALUMINIUM PHOSPHIDE NEUROTOXICITY IN ALBINO RAT"* has been carried out by ***KUNWAR ASIF*** under my guidance. This work is original and has not been submitted so far, in part or full for any other degree or diploma in this or any other university. He is allowed to submit the work for the award of the degree of ***Doctor of Philosophy in Chemistry*** of the Aligarh Muslim University Aligarh.

(Prof. Shamim Jahan Rizvi)
Co-supervisor

Abbreviations

ACh	:	Acetylcholine
AChE	:	Acetylcholinesterase
ad libitum	:	(L) at one's pleasure
ANOVA	:	Analysis of Variance
AlP	:	Aluminium Phosphide
BS	:	Brain Stem
CAT	:	Catalase
CBL	:	Cerebellum
CBM	:	Cerebrum
CNS	:	Central Nervous System
DNA	:	Deoxyribonucleic Acid
DA	:	Dopamine
DN	:	Delayed Neurotoxicity
et al.	:	(L) et alii and others
GR	:	Glutathione Reductase
GSH	:	Glutathione (Reduced) (Free Sulfhydryl Groups)
GSHPx (GPx)	:	Glutathione Peroxidase
GSSG	:	Glutathione (Oxidized)
GST	:	Glutathione-S-Transferase
LPO	:	Lipid peroxidation
LHPO	:	Lipid hydroperoxidation
µ	:	Micron
M	:	Molar
MAO	:	Monoamine Oxidase
MDA	:	Malondialdehyde
N	:	Normality
NE	:	Norepinephrine
OD	:	Optical Density
RNA	:	Ribonucleic acid
SE	:	Standard Error
SOD	:	Superoxide Dismutase
SPC	:	Spinal Cord
T-SH	:	Total Sulhydryl Group
O.P.	:	Organophosphate
I U	:	International Unit

Chemicals Used

(All Chemicals used were of Analytic Grade)

1. Absolute alcohol
2. Acetone
3. Acetyl cholinethio iodide
4. Acetic Acid (Glacial)
5. Bovine Serum Albumin (BSA)
6. Butanol (normal)
7. Chlorodinitrobenzene (CDNB)
8. Chloroform ($CHCl_3$)
9. Copper Sulphate ($CuSO_4$)
10. Dinitrothiobis Benzoic Acid (DTNB)
11. Diphenylamine Reagent
12. Eserine Sulphate
13. Ethelinediaminetetra Acetic Acid (EDTA)
14. Foline-Ciocaltau Reagent
15. Ferric Chloride ($FeCl_3$)
16. Ferrous Ammonium Sulphate
17. Glutathione (GSH)
19. Hydrogen Peroxide (H_2O_2)
20. Hydroxylamine hydrochloride
21. Hydrochloric Acid (HCl)
22. Imidazole (glyoxaline)
23. Methanol (Absolute)
24. N-Ethyl maleimide (NEM)
25. NADPH (Sodium Salt)
26. Perchloric Acid (70%)
27. Orcinol Reagent
28. Potassium Chloride (KCl)
29. Potassium Thiocyanate (KSCN)

30. Phosphate Buffer.
31. Pyrogallol
32. Sodium Azide (NaN_3)
33. Sodium Hydroxide, Sodium chloride, Sodium carbonate, Sodium acetate, Sodium pot. tarterate, Sodium dodecyl Slphate (Laury Sulphate) and Succinic acid.
34. Sulphosalicylic Acid (SSA)
35. Thiobarbuteric Acid (TBA)
36. Trichloroacetic Acid (TCA)
37. Tris-HCl Buffer

Contents

Tables and Figures

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Figures

ACKNOWLEDGEMENTS

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Man is instinctively born inquisitive and his powerful instinct has been to know more and more about the world and his own body. Modern medical science is the sum total of the continuity of researches made over hundreds of years by the most eminent men of science. These great masters, who were truly the pioneers in their fields, delved deep into mysterious of the human body. Blessed indeed are those pioneers who for a batter and healthier living of man, have been whole-heartedly dedicating their lives, at the cost of their personnel comfort and easy to the fulfilment of the mission of their lives. One of such living pioneer is Prof.Dr. Mahdi Hasan (FNA) founder director, INTERDISCIPLINARY BRAIN RESEARCH CENTRE (IBRC), J.N.Medical College, A.M.U., Aligarh, who is respectfully regarded as an intellectual giant amongst one of the most eminent man of science, not only in India but also in the most progressive countries of world where science have made great strides. His masterly grasp of the subject under study, his efficiency as one of the most competent and successful teacher and his thought-provoking researches have reverently endeared him to one and all. It has been indeed my sheer good luck to learn a little from him and to have been blessed with his guidance based on his vast knowledge and wide experience of three decades. But for his unstained guidance and almost fatherly help, it would have not been possible for me to submit this thesis, because his timely and spontaneous encouragement came to my rescue many a time when despondency loomed large over me.

To **Dr. Syed Ashfaq Nabi**, Reader, Department of Chemistry, A.M.U., Aligarh. I owe a deep sense of gratitude for his assistance and guidance as supervisor for the completion of this research work. His dynamic personality,

ability, knowledge and experience as scientist have been of a greater assessment to lighten my path. I honestly admit it that as it is my pride of being his pupil. I shall always be obliged and grateful to him throughout my life and future generations.

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I convey my respectful thanks to my grand mother and uncles for their blessings and affectionate exhorations which inspired and invigorated me to complete this thesis.

I shall be failing in my duties, if I forget to express my thanks to my best friend and research colleague **Mr. Ishrat Jamil**, who inspired and assisted me to join the **I.B.R.C.**, he always proved himself as a pathfinder to me in research and in practical life. His versatile personality , caliber, modesty and way of handling the matter will always remain a matter of pride to me, to I.B.R.C. and to A.M.U.

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Finally, I fall short of adequate words in expressing my thanks to my **fiancee** for her moral support and encouragement.

(Kunwar Asif)

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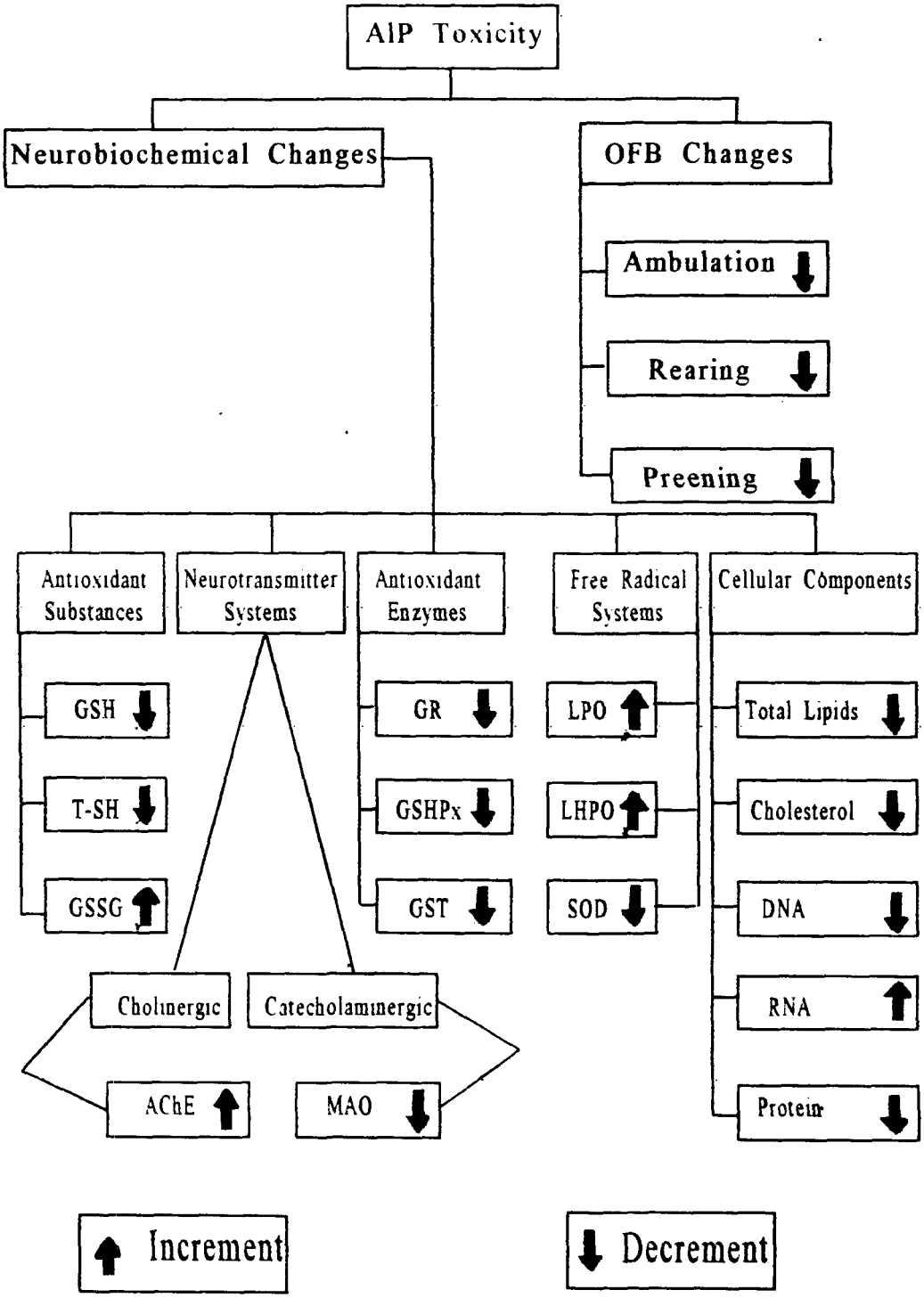


REVIEW OF LITERATURE

Review of Literature

2.1 Poison and Poisoning: Historical Back Ground:

Poisons and poisonous substances that played a dominant role in the affairs of mankind, producing serious injury or death, have been documented from the times of ancient Egyption magicians, physicians and priests. These are first documented recognition of the existance of these poisons. The medical records of ancient Egypt include a practicing physician's formullary containing about 260 prescriptions, some of which are for various kinds of animal stings, including the posionous puffer fish. The Bible and Talmudic records document the toxic effects of dinoflagelates (a group of algae). Other records list some 760 medicinal plants and mention venomous animals and antidotes for bites and stings, and charms against snake poison (Halstead B.W., 1978).

In ancient China, toxicology was a comparatively well developed science. The ancient Greeks were probably the first to dissociate medicine from magic. Hippocrates, while introducing rational medicine

described a number of poisons. Mithradates VI, king of Pontus in the 2nd centruy B.C., is said to have been one of the first to study intensively the art of poisoning and the preparation of antidotes to poisons.

During the middle ages, poisoning became a very popular way to dealing with personal and political problems. Individuals skilled in the art of poisoning gained notoriety and in some cases, great financial reward. The French physician, Jacques Grevin published his book DEUX LIVERS DES VENINS in 1568. In 1702, the British physician Robert Mead published his "Mechanical taconite of poisons". Important toxicological contribution of the French physician M.I.B. Orifila (1787-1853), who has ocurred the name of father of modern experimental toxicology. His Traite Detoxicologie first published in 1814, went through many editions and translations.

Experimental research based on sophisticated analytical procedures accelerated in the early 20th century. During World War II, environmental and biological poisons become medically important in many military operations. A rapidly expanding world

population in the Post War Years has increased the use of various pesticides in a continuing effort to feed the milions. This has increased the risks of toxicity, both intentional and accidental, especially in the underdeveloped countries like ours, where illiteracy, poverty and lack of infra structural facilities combine to take a heavy toll.

2.2 Aluminium phosphide : Nature of poison

Aluminum phosphide (AlP_3 Celphos, Quickphos, Alphos, etc.), is a solid fumigant. AlP is commercially availabel as solid pellets, each of 3.0g, in air sealed metal tubes.

Ingredient of pellets :-

i. 56% PH_3 (Active ingredient)

ii. 44% Ammonium carbonate

On contact with moisture or hydrochloric acid, the pellets rapidly release phosphine (PH_3) gas which is the active agent, as well as CO_2 and NH_3. Each 3.0g pellet of AIP can liberate 1.0 g of PH_3. The human toxicity is due to inhalation of phosphine from the fumigated

grain or after ingestion of AIP.

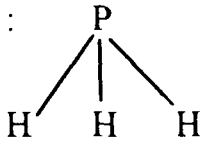
$$AlP + 3H_2O \rightarrow Al(OH)_3 + PH_3$$

$$AlP + 3HCl \rightarrow AlCl_3 + PH_3$$

2.3 Physico-Chemical Properties of AIP:

Common Name	:	Quickphos (Celphos)
Chemical name	:	Aluminum phosphide
Active Ingredient	:	Phosphine
Structural formmula	:	AlP (Al≡P)
Molecular Weight	:	57.00

2.4 Properties of Phosphine (PH_3)

Structure Formula : 

Molecular Weight	:	34.00
Solubility	:	Soluble in water and inorganic solvents

2.5 Lethal Dose of PH_3

The lethal dose for 70 kg human being is 150-500 mg determined recently in most cases of acute intoxication reported. Phosphine is dangerous to human life at a concentration between 400-600 ppm. At 1000 ppm, it is rapidly lethal (WHO, 1988).

A dose of less than 0.5 g of AlP has been reported to be lethal for an adult individual (Gosseline et al. 1984). However, other authors have quoted doses as low as 0.01. g (Sidney, 1980).

2.6. Aluminium Phosphide Poisoning in Humans :

The 1st reported case of toxicity due to AlP ingestion was by Zipf et al., (1967). Prior to this , toxicity due to inhalation of PH_3 has been reported. Gessner (1937) reported 12 cases of illness begining with nausea and including one death in a house adjacent to a warehouse in which bags of AlP were stored. As recently as 1980, 31 crew members abroad a freighter carrying fumigated grain, after inhaling phosphine, one child was died (Wilson et al. 1980).

In India, the 1st case of AlP toxicity was reported in 1981, and since then it has assumed epidemic proportions in the entire wheat belt of North India (Bajaj and Wasir, 1988). This is clearly the case in Haryana (Siwach et al., 1988), Chandigarh (Singh et al., 1985), Western U.P. (Ram et al., 1988), M.P. (Caha et al., 1988) and Rajasthan (Saraswat et al., 1985).

The true magnitude of the problem is not completely known. Many deaths from AlP poisoning occur before the victim can be hospitalized, and practically no reports are available from non-teaching hospitals, peripheral health centres and private practitioners. Even teaching hospitals provide mope reports. It is estimated that less than 10-15 % of patients reaching a medical college hospital have actually been reported, and these in turn form only about 5% of the total victims (Bajaj & Wasir 1990). Even in 1988, it had been suggested that the number of deaths, exceeded the number of reported casualties in the Bhopal gas tragedy (Kobra and Narayanan ,1988). During Iran and Iraq War, in 1985, the dangerous biological bombs used by Iraq released PH_3 gas. It is clear that AlP

ingestion is now the single most frequent mode of suicide.

The poison is usually taken by young adults, mostly teenagers by suicidal intent or occasionally by accident.

2.7 Clinical Manifestations of AlP Toxicity: Sign and Symptoms

The sign, and symptoms of AlP toxicity depend on the dose and severity of poisoning. Mild poisoning manifests as epigastric burning and nausea, with tachycardia and atrial ectopy. Patients with moderate to severe toxicity were found with vomiting and epigastric burning and are usually in cardiovascular shock with thready pulse, cold extremities and restlessness (Bajaj et al., 1988).

Phosphine is a systemic poison and it affects the cardiovascular, gastrointestinal, renal, respiratory, central nervous system and hepatobiliary system.

Feature of above systems

Cardiovascular : The heart is involed in nearly 50% of cases.

(i) Elevation of serum levels of cardiac enzyme CPK-MB and LHD (Bajaj et al., 1988).

(ii) Hypokinesia of the left ventricle, with decreased ejection fraction in the first 4 days, hypomagnesemia (Mg depletion). Bajaj et al., 1988).

(iii) Histopathological studies of myocardial tissues show myocardosis and inflammatory cells (Siwach et al., 1988).

(iv) ECG changes comprise ST-T changes (elevation/ depression, caving). Circulation, disturbances Sino-Attial, atrio-ventricular, bundle branch blocks and rhythm disturbances (ventricular and superventricular) [Katira et al. 1990] as well as atrial infarction (Jain et al., 1985).

Respiratory: Adult respiratory distress syndrome has been reported in severe cases cough, dyspnoea and cyanosis (Chough et al., 1988).

Renal : Renal failure alongwith hepatitis and bleeding diathesis has been reported (Gupta et al., 1990).

Hepatobiliary: Jaundice and hepatitis.

CNS: Inhaled phosphine is absorbed through the lungs and reaches the nervous system, and liver thereby producing neurological and hepatic symptoms (Childs and Choates, 1971). Symptoms such as headache, dizziness, altered mental state, restlessness without alteration in conciousness, convulsions, acute hypotoxic encephalopathy and coma, follow soon.

Gastrointestinal: Common findings are : nausea , vomiting, diarrhea etc.

2.8 Detection of the Poison

Phosphine poisoning may be detected by means of a simple test using filter paper impregnated with fresh silver nitrate (0.1 M) solution (Mittal et al., 1992). The test is performed with the help of gastic aspirate. The $AgNO_3$ paper test is based on the capacity of PH_3 to reduce $AgNO_3$ which gives a black precipitate.

$$PH_3 + 8\ AgNO_3 + 4\ H_2O \longrightarrow \underset{\text{Black}}{8\ Ag} + H_3PO_4 + 8\ HNO_3$$

The senstivity of this test is high and it affords a ready means of confirming poisoning by AlP, especially

when the history is not forthcoming.

2.9 Mechanism of Action of Phosphine:

The mechanism of action of PH_3 is not well understood. The lethal properties of AlP are due entirely to the liberation of PH_3. The mode of action of PH_3 has been studied in numerous animal models. It has been shown by various investigators to act on the respiratory chain. PH_3 liberated energy during the oxidation of fatty acids and amino acids and nearly all of that released from the oxidation of carbohydrates is made available within the mitochondria as reducing equivalents (-H or electron). Mitochondria contain the series of catalysts known as the respiratory chain that collect and transport reducing equivalents and direct them to their final reaction with oxegen to form water.

Lipoate ⟶ NADH ⟶ NADH-Q-reductase ⟶ Q ⟶ Cyt reductase ⟶ Cyt C ⟶ Cyt Oxidase ⟶ O_2

(Components of the respiratory chain in mitochondria)

Cyt : Cytochrome ; Q : Ubiquinone

The terminal cyt. is responsible for the final combination of reducing equivalents with molecular oxygen. This is the only irreversible reaction in the respiratory chain, and gives direction to the movement of reducing equivalents in the respiratory chain, and to the production of ATP.

Oxidation and phosphorylation are tightly coupled. Nakakita et al. (1971) carried out in vitro studies in rat liver mitochondria showed phosphine to be potent inhibitor of ADP and Ion - stimulated respiration. However, no attempt was made to identify the target site (Nakakita et al., 1971). Chefurka et al. (1976) investigated the basis of the respiratory inhibition of phosphine in mitochondria from mouse liver and housfly flight muscles . Their conclusion was that phosphine is an inhibitor of state 3 and 4 respiratory activity by virtue of its direct inhibition of electron transport due to interaction with cytochrome oxidase.

The inhibition kinetics suggested non competitive inhibition of this site; oxidative phosphorylation was not

affected. Chefurka et al (1976). Studied the nature of the interaction between phosphine and purified cytochrome oxidase by absorption spectrometry and circular dichroism and noted a comformational change in the haem moiety of cytochrome oxidase. They concluded that cyt. was the major site of action of phosphine (Khashi and Chefurka, 1976). In the event of inhibition of cytochrome oxidase, oxygen undergoes an alternative pathway of reduction resulting in formation of free radicals. (Fridovich, 1978b).

Price (1980) studied the inhibition of cytochrome C-oxidase by phosphine in vitro in both susceptible and resistant strains of R. dominica but found little inhibition of cytochrome oxidase. The toxicity of phosphine to insects is oxygen dependent (Bond et al., 1967). In the absence of oxygen, phosphine is virtually nontoxic and is not absorbed to any appreciable extent. Price et al. (1982) and Price and Dance (1983) suggested that this may be due to the effect of phosphine on the haem containing enzyme catalase. Catalase is an enzyme which reduces hydrogen peroxide to water in the body; it is suggested that it also plays a vital role in the

detoxification of free oxygen radicals (Master and Holmes, 1976). Bolter and Chefurka (1989) observed that phosphine reduces cytochrome C-oxidase but none of the other cytochromes in the electron transport chain.

Thus whether by inhibition of cytochrome oxidase or of catalase, phosphine toxicity would be expecte to lead to over production of free oxygen radicals.

2.10 Free Radicals:

Electrons in the atoms are present in orbitals, each of which can hold a maximum of two electrons, spinning in opposite direction. A free radical is defined as any atom or group of atoms, or molecule capable of independent existance in a particular state with one or more unpaired electrons in the outer orbital. These are a highly unstable and reactive species (Del Maestro, 1980). Free radical reactivity is accounted for by the strong tendency of the unpaired electron to interact with other electrons to form an electron pair and thus a chemical bond. Free radicals tend to give rise to chain reactions, which may be conveniently divided into three stages (1) Initiation (ii) propagation (iii) termination.

i. **Initiation** reaction by which the free radicals are formed (Pryor, 1966).

ii. **Propagation** reaction in which the free radical chain transfer reaction.

iii. **Termination** reactions resulting in removal of free radicals from the propagation pool.

The presence of an unpaired single electron in its outer orbital is conventionally represented by a superscript dot, $\dot{R}$.

2.11 Oxygen Free Radicals:

Molecular oxygen in its ground state is a bi radical possessing two unpaired electrons in its outer orbital with parallel electron spin (Tabue, 1965; Ogryzlo, 1978). This arrangement prevents the direct addition of a pair of electrons (which would have one parallel and one antiparallel spin) to the molecule necessitating an electron spin inversion before an oxidation reaction is possible. The spin inversion is a slow process compared to the life-time of collisional complexes are relatively weak oxidant (Toube, 1965), the restriction in the oxidising

when it under goes univalent electron reduction along with electron spin inversion, results in the formation of toxic intermediates of the free oxygen radicals.

During oxidative phosphorylation, the mitochondrial cytochrome oxidase enzyme system links production of adenosine triphosphate to controlled tetravalent reduction of molecular oxygen (O_2) to water (Antonim, et al. 1979). In this process, the partially reduced oxyen free radical intermediates remain lighlty bound to the active sites of the enzyme and pose no threat to the cells.

However, in the presence of intercellular oxygen, this reduction of oxygen is frequently incomplete and the univalent reduction pathway predominates over the tetravalent pathway leading to the inadvertant production of toxic intermediates oxygen species, superoxide anion radial ($O^{\cdot}_2$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2).

$$O_2 \xrightarrow{e^-} O^{\cdot}_2 \xrightarrow{e^- + 2H^+} H_2O_2 \xrightarrow[H_2O]{e^- + 2H^+} H_2O \xrightarrow{e^- + 2H^+}$$

$$OH^- \xrightarrow{e^- + 2H^+} H_2O$$

2.12 Biological Sources of Free Radicals:

Many enzymes in the body produce univalent production of molecular oxygen leading to the formation of toxic free radicals. In addition, these reactive metabolites may arise form the action of various environmental agents. Possible sources of free radicals are indicated in the following table (Sinclair et al. 1991).

Source	Mechanism	Example
Cellular metabolism	Oxyhaemoglobin	
	Enzymic activity	NADPH oxide
	Arachidonic. Acid metabolism and PG synthesis.	Xanthine oxidase
	Mitochondrial electron transport Auto-oxidation	Endoplasmic reticular oxidation
Environmental		Catecholamines Reduced Riboflavin Meluminthiols Flavin derivatives
	Drugs. Pesticides Tobacco smoke Radiation	Paracetamol, Halothane Paraquat etc.

2.13 Biological Consequences of Free Radical Damage:

Free radicals mediate injury at several sites including cell membrane lipids, the sulfhydryl group of protein and nucleotides of DNA (Llebanoff, 1988; Slater, 1984; Inlay and Linn, 1988; Dormandy, 1988). This injury occures through several mechanisms. (Del Maestro, 1980).

i. Peroxidation of polyusaturated fatty acids and the subsequent disruption of cell and organelle membranes.

ii. Lysosomal membrane disruption resulting in the autophagocytic vacuole formation.

iii. Protein damage leading to fragmentation, cross linking and aggregations of proteins.

iv. DNA degradation resulting in mutations and potential neoplastic transformation.

v. Membrane phospholipase activation leading to release of prostaglandins and various endoperoxides.

vi. Noxious products of lipid peroxidation may be transferred by the circulation to distant sites where they may provoke further damage.

2.14 Protective Mechanism Against Free Radical Damage:

Normally, a small amount of free radicals is produced in the body with its attendant risks of cell injury. The free radicals may decay spontaneously, but several cellular mechanisms exist for both enzymatic protection and non enzymatic antioxidant mediated protection.

2.14.1 Enzymatic Protection:

i. Superoxide Dismutase (SOD)

This enzyme converts superoxide anion (O_2^-) to H_2O_2

$$O_2^- + O_2^- + 2H + \xrightarrow{SOD} H_2O_2 + O_2$$

SOD increase the rate of intracellular dismutation of superoxide by a factor of 10^9 (Fridorich, 1978a). The, acceleration of this reaction ensures that no superoxide anion is availabe to react with H_2O to form hydroxyl

radical through the metal catalysed Fenton reaction. The cells are capable of increasing the synthesis of SOD in response to hyperoxident stress (Firdorich, 1983).

Two enzyme systems exist to catalyse the breakdown of H_2O_2 produced by the univalent reduction of superoxide anion (Roose et al., 1980; Chance et al., 1979).

ii. Glutathione Peroxidase (GSHPx):

At low concentrations, most of the H_2O_2 is removed by reaction with reduced glutathione (GSH). GSHPX is a selenium dependent enzyme present mainly in the cytoplasm. It catalyzes the transfer of hydrogen from the sulfhydryl (-SH) to a hydroxyl radical or to H_2O_2.

$$2OH^{\cdot} + GSH \longrightarrow 2H_2O + GSSG$$

(Oxidized glutathione)

$$H_2O_2 + 2\ GSH \longrightarrow 2H_2O + GSSG$$

The enzyme glutathion reductase (GR) catalyzes the regeneration of reduced glutathione (GSH) from GSSG by using NADPH formed by the hexose monophosphate shunt pathway. Glutathione is a key factor in the

detoxification of reactive oxygen intermediates and electrophilic metabolites (Curello et al., 1987).

iii. Catalase : At high concentration of H_2O_2, which is mainly present in the peroxisomes, catalase becomes important.

$$2H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2H_2O$$

2.14.2 Nonezymatic Protection: Apart from enzymes, endogenous as well as exogeneus antioxidants may either block the initiation of free radicals or inactive them.

2.15 α-Tocopherol (Vitamin E):

About thirty years have passed since the existence of vitamin E was recognized. Alpha tocopherol the substance with highest known vitamin E activity was synthesised about twenty years back. The principal group of compounds having vitamin E activity are the tocopherols. The seven tocopherols have been found to occur naturally.

The biopotencies of various tocopherols differ somewhat, depending on the assay criteria and animals used. α–Tocopherol has the greatest biological activity, however. This could be a reflection of its greater susceptibility for methylation. Such methylation will prevent side reactions and lower the oxidation-reduction potential of hydroquinone-quinone system. The features of tocopherol chemistry that appear most salient to possible biological function are its lipid solubility and oxidation properties. They play a physiological antioxidant role (potentiated by ubiquinone Q10) at the cellular level by counter acting lipid peroxidaton increasing membrane phospholipids. Vitamin E acts as a H^+ donor in Krebs Cycle, between the steps of flavin coenzymes (FMH and FAD) and the cytochrome system (Butturini, et al. 1955).

The identification of vitamin E as a fat-soluble vitamin, its occurence in vegetable oils, its storage in association with body lipids and its possible function as a biological antioxidant suggest that close relationship exists between vitamin E and various phases of lipid metabolism. When vitamin E is deficient or completely

lacking, there is uncoupling of phospholipids and proteins, necessary for formation of cell membrane. In studies in humans, the administration of tocopherol to healthy male subjects resulted in increase in plasma cholesterol values (Gray and Lon, 1958.) Grey (1959) found levels of phospholipids and cholesterol esters in livers of rats fed 100 mg α-tocopherol acetate daily when compared from unsupplemented, rats presumably due to increased hepatic lipid synthesis in the supplemented animals.

After the poineering works of Kudrjashow (1937) and Davis and Moore (1941) an appreciable number of scientists have been dealing with the relationship between vitamin E and lipid peroxidation. The contribution of Tappel's laboratory has generally been recognized (Tappel, 1962, 1972, 1973). The increase in lipid peroxidation resulting from E-avitaminosis was proved beyond question. The activation of lipid peroxidation increases the requirement of vitamin E (Willing, 1970, 1972). Synthetic antioxidants prevent symptoms of E-avitaminosis, though not completely (Rods, 1967; Tappel, 1972). The toxic action of lipid

peroxidation products (Holman and Greenberg, 1958) is usually inhibited by α–Tocopherol (Kokathur et al., 1966; Privett and Cortesi, 1972).

α-Tocopherol is a powerful chain breaking inhibitor of lipid peroxidation. Vitamin E acts in the lipid phase to refuse the lipid peroxy radicals (Clavce, et al., 1979; Halliwell and Gutteridge, 1985).

2.16 Free Radicals in Poisoning:

The central role of free radicals in toxicity due to many chemicals is established. For example, the toxicity of the herbicide paraquat is due to enzymatic reduction to paraquat-pyridinyl cation radical and its reoxidation by hydroxyl redicals and by the Fenton and Heber-Weiss reactions (Osheroff, et al., 1985)

In AlP poisoning, the inhibition of either cytochrome oxidase or catalase would be expected to give rise to an abundance of $0^{\cdot}_{2}$ free radicals, (Clough, et al., 1992).

Although abundant literature is available on the effects of AlP on different organ systems, the neurotoxic

efect of AlP on different regions of brain has not been reported so far.

2.17 Treatment of AlP Poisoning:

To date, there is no known specific antidote for AlP poisoning, therefore, the therapeutic part is mostly restricted towards symptomatic treatment and maintenance of vitals.

The manual of Indian standard code for safety for AlP poisoning suggests :

i. Induction of vomiting by administration of 0.2% $CuSO_4$ solution which acts as an emetic and also forms insoluble cupric phosphide which can be taken out by gastric lavage with 1:5000 $KMNO_4$ solution.

ii. 25.0ml of milk of magnesia or white beaten of 2 or 3 eggs should be given.

iii. Vasopressors (Dopamine, noradrenaline) for maintenance of blood pressure.

iv. Urinary pH is made alkaline by giving sodium carobnate (Na_2CO_3)

v. Pulmonary oedema is treated on standard lines. Intranasal oxygen, steroids and hypertonic solution of glucose may be given.

vi. Haemodialyais may be required for renal shutdown which may be an outcome of shock syndrome due to direct tissue toxicity.

In the present study, effect of α-Tocophrol (vitamin E) was studied in different regions of rat brain. To date no report is available on the antioxidant effect of vitaimin E on the treatment of CNS toxicity due to AlP inhalation.

2.18 The Lipids:

Lipids are essential components of all cell membranes and are involved in numerous biological process they are also a major components of several critical enzyme systems, include a potent class of hormones, and are also implicated in the cellular transport of some components (Spectator and

Brenneman, 1973). Tumors of cancer require lipids so that membrane synthesis can proceed at a significant rate to permit rapid growth. If the availability of lipids is sufficiently reduced then the growth of tumor is also slowed down (Spectator and Brenneman, 1973). Lipids are the most concentrated source of energy to the organism, they are stored in a relatively water free state in the tissues, in contrast to carbohydrates which were heavily hydrated. The lipid depots serve as a reservoir of energy, available in times of restricted nutrition for the operation of the numerous endogenic processes essential for maintenance of life. Lipids work as insulators of heat. The subscutaneous lipid depots also insulate against mechanical trauma (White et al., 1978). Lipids are chemically triacylglycerol in human adipose tissue (99%).

The quantity of body lipids is increased by excessive nurtrition, and conversely, during periods of prolonged fasting the amount of body lipid decreases. Fatty acids are used by the body tissues as their prime energy source (Fritz, 1961) and direct evidence for the up take and utilization of fatty acids from the circulatory

blood by individual organs and tissues in the fasting state has been obtained (Shipp et al., 1961; and Gousios et al., 1963). Depot lipid is continously being mobilized, new lipid is continously being deposited, and the constancy of the quantity of depot lipid is the result of a relatively precise adjustment of the rates of these two processes. Almost 10% of the fatty acids in the depot lipid is replaced daily by new fatty acids in the body.

2.18.1 Brain Lipids:

"There is no other organ in the body that contians such a high proportion of lipids as the brain".

Studies of lipids in the nervous system form an important part of neurochemical investigations. Among various body organs, the brain is one of the richest in lipids, comprising over half of the total dry weight (Brante, 1949: Balakrishnan et al., 1961: and Suzuiki, 1981). It contains a unique structure, the myelin sheath, which have the highest lipid concentration of any normal tissue or subcellular components, except for adipose tissue, and which has been the subject of intensive and extensive studies in recent years. Myelin is present in all parts of the nervous system but is more concentrated

in areas composed mainly of fiber tracts, such as the white matter of brain and spinal cord and in peripheral nerve trunks, such as sciatic nerve. The abundant lipids of CNS are located in both cellular and subcellular membranes and in the myelin sheath. Different types of membranes accumulate different types of lipids (Horrocks et al., 1975). Mammalian brain white matter contains about 50% myelin on a dry weight basis. Even in the whole brain of an adult rat, myelin is about 25% of dry weight and accounts for more than 40% of the brain lipid (Northon and Poduslo, 1973). Early analysis of white matter revealed that cholesterol, sphingomyelin, and cerebroside were present in larger amounts than in the grey matter (Johnson et al., 1948). Cumings (1953 and 1955) compared the lipids of demyelinated lesions in multiple sclerosis with those of normal areas of the brain and found a decrease in sphingomyelin, cerebroside, and free cholesterol. Similar findings were made in demyelination resulting from Walarian degeneration of the peripheral nerve (Rossiter, 1961 and Berry et al., 1965). The rapidly increased myelin content is closely related with increase in brain weight (Smith et al., 1983).

Waelsch, Sperry and Stayanoff (1941) have studied that after birth lipid is deposited in the brain as a result of two processes, (a) growth and (b) myelination. Immediately after birth and before myelination is complete there is active deposition of brain lipids (Fries, Changus and Chaikoff, 1940). The important lipids of the CNS are chlesterol, cerebrosides, phospholipids, lecithin,sphingomyelin & Cephalin (Johnson, et al. 1948). Galli et al. (1970) indicated that rat brain may have sphingomyelin levels as high as in human brain. About 50% of all lipid in white matter or 30% of total brain lipid, has been estimated to belong to the myelin sheaths in rat brain. The complete lipid analysis of myelin from different species has been published by various workers (Autilio et al., 1964; Eichberg et al., 1964; and O' Brein, 1965). the most rapid increase in lipid content of brain begins after the periods of greatest increase of DNA and protein. Changes in specific lipids have been well documented (Rouser and Yamamoto, 1969 and Wells and Dittmer, 1967). Changes in fatty acid concentration and degree of saturation have been determined for several groups of lipids (Rouser and Yamamoto, 1969 and O'Brien, 1979).

Studies from this laboratory have shown that pesticides perturb the levels of total lipids in the rat CNS (Tayyaba and Hasan, 1980; Islam et al., 1983 Tayyaba and Hasan, 1985 Hasan and Khan, 1985 and Naqvi et al., 1988; Gupta and Hasan., 1988).

The available literature on different lipids indicate that the knowledge of pesticide toxicity on brain lipids is inadequate. The different parts of the brain show regional variations in the lipid contents. Since the brain is a heterogeneous organ composed of many structural and functional components with markedly differet levels of functional and metabolic activity, it is reasonable to investigate the neurotoxicants' influence on discrete brain areas. The present study deals with the effect of phosphine inhalation on different lipid levels in various regions of CNS of the rat brain

2.19 Cholesterol:

The myelin of mammalian CNS is composed of 25 to 28% cholesterol, 40 to 45% phospholipid and 27 to 30% galactolipids. Brante (1949) estimated that myelin sheath lipids were 25% cholesterol, 46%

phospholipides and 29% galactolipids. The data for lipid composition are expressed in mole percent. Most of the preparations analysed so far contain cholesterol, phospholipid and galactolipid in molar ratios ranging from 2: 2: 1 to 4: 3: 2. Thus, cholesterol constitutes the largest proportion of lipid molecules in myelin. It contains approximately 25% of myelin lipid by weight (Soto et al., 1966 and Cuzner., 1965). The structural matrix of cell membranes is a lipid bilayer with variable amounts of cholesterol and glycolipids (Tanford, 1978).

Cholesterol, is the only sterol present in normal adult brain in significant amounts. The alcohol group (-OH) at position 3 may be esterified with a long-chain fatty acid. Esterified cholesterol is present in normal brain only in very low concentrations. In adult brain it is in unesterified form (Davison, 1965). Unesterified cholesterol has been suggested as a lipid that is characteristic of myelin sheaths. It occurs in white matter in amounts greatly exceeding those in grey matter (Johnson, 1949 and Brante, 1949). Along with cholesterol in CNS, there occur very small amounts of other sterol (upto 1%) (Cook, 1958). In rat brain, total

levels of sterol esters increase from birth to 40 days (Eto and Suzuki, 1972). Kritchevsky and Holmes (1962) found varying amounts of the sterol in the newborn rat brain,. Recently, Fumagalli and Paoletti (1963) and Fumagalli et al. (1964) reported that desmosterol accounted for upto 7% of the human and rat fetal and neonatal brain sterol content. Desmosterol which is the immediate metabolic precursor of cholesterol (Fish et al., 1962) and has an additional double bond at C_{24}, is present in normal developing brain in measurable amounts just prior to mydination (Kritchevsky et al., 1965 and Paoletti et al., 1965) and also in the myelin sheath itself in the early stage of myelination (Smith, et al., 1967). Laatsch, et al. (1962) demonstrated that cholesterol accounts for 18-20% of the dry weight of the myelin fraction and that about 70% of the total brain cholesterol is present in the myelin. Cholesterol accounts for about 10% of dry weight of the brain in contrast to less than 11% found in most other organs.

Biosynthesis of cholesterol in brain is most rapid during the period of active mydination. but adult brain retains the capacity to synthesize cholesterol when

precursors such as acetate or mevalonate are available. Acetate and its precursors are transformed through mevalonic acid to cholesterol. The adult human brain contains 25g of cholesterol, but this amount at birth is only 2g (Waelsch et al., 1940 and 1941). On pH 7.2 cholesterol ester hydrolase is one of three such hydrolases in brain that can be distinguished by their pH optima and response to detergents (Eto and Suzuki, 1973 and Igarashi and Suzuki (1977).

Although most of the cholesterol in brain appears to be synthesized from endogenous precursors, experimental evidence indicates that a small amount of systematically injected cholesterol can be taken up intact and that the rate of uptake is greatest when the rate of cholesterol deposited in brain is most rapid, i.e., during active myelination (Dobbing, 1963). Once deposited in brain, cholesterol, particularly that incorporated into myelin, is relatively inert metabolically (Davison et al., 1958 and Khan and Folch, 1967). Further studies of incorporation of labelled cholesterol or labelled acetate into brain tissue indicate that the cholesterol of adult brain is relatively inert (Waslsch et al., 1940; Bloch et

al., 1943; Syere et al., 1950; and Van Bruggen et al., 1953). After intracereberal injection of labelled cholesterol acetate in rats, however, some label is incorporated into cholesterol and appears to remain there indefinitely (Nicholas and Thomas, 1959). Paoletti (1971) has substantiated the obervation that microsomes are the subcellular sitesof brain cholesterol biosynthesis.

Earlier findings from this laboratory have shown alterations in the levels of cholesterol in discrete brain areas following chemical stress (Tayyaba and Hasan, 1980; Islam et al., 1983; Hasan and Khan, 1985; Tayyaba and Hasan, 1985; Vadhva and Hasan, 1985; Naqvi et al., 1988 and Gupta and Hasan, 1988; and 1992).

Till date, no attempt has been made to evaluate the effect of phosphine in the various regions of the rat brain and spinal cord cholesterol. Therefore, it would be of interest to estimate the levels of cholesterol quantitatively after the administration of AlP solution by gavage.

2.20 Lipid Peroxidation:

The lipids in membranes of cells from higher organisms contain large numbers of polyunsaturated fatty acid side-chains. Such fatty acids are prone to undergo a process known as "lipid peroxidation", which involves the generation of carbon radicals, followed by production of peroxide radicals (Sohail, 1981). Lipid peroxidation has been broadly defined as the oxidative deterioration of polyunsaturated lipids and involved in many disease processes and chemical toxicities (Tapple, 1973 and Tappel and Dillard, 1981). Lipid peroxidation is found to be affected in certain clinical disorders such as under nutrition, cancer, aging, hypoxia etc. It has been claimed that lipid peroxidation in vivo has been of basic importance in aging, damage to cells by air pollution, toxic chemicals and in oxygen toxicity (Tapple, 1970). It is believed that lipid peroxidation of biomembranes is one mechanism whereby a foreign chemical may be toxic to cells, and this has evoked considerable interest in understanding the mechanism of this phenomenon in various biological systems (Bus and Gibson, 1979 Msayuki et al., 1996). According to Demopoulos et al.,

(1979) the loss of membrane integrity during pathological free radical mechanism leading to lipid peroxidation and degeneration of phospholipids are important factors which irreversibly damage brain cells in ischaemic and other adverse conditions. The rate of lipid peroxidation is also highly catastrophic to the integrity of cellular membranes and to membrane bound enzymes (Recknagel and Glence, 1977 and Zebra, et al., 1990). It is appropriate to recall here that, as well as being destructive at higher concentrations, lipid peroxides also appear to have an essential enzyme activating role in the arachidonate metabolizing pathways (Helmer and Lands, 1980; reviewed by Clevland, 1984). Free radicals have long been suspected as intermediates of biological oxidative processes. They are highly reactive transient chemical intermediates, the concentration of which is increased by high energy irradiation to the oxygen radicals themselves, certain products of radical induced lipid peroxidation, including a series of aldehyde, may be toxic to invading organisms and host cells (Morel et al., 1983). Being much more stable than free radical, these toxic compounds can

cause injury some distance from the site of radical generation. Kartha and Krishnamurthy (1978) reported that among the diferent tissues from normal rats, the brain showed a considerably high degree of peroxidation, while the homogenate of other body parts showed comparatively low lipid peroxidation. When lipids react with oxygen radicals they undergo a series of molecular rearrangements termed peroxidation and form a series of oxidation derivatives, including lipid hydroperoxides and aldehydes (Esterbauer, 1982 and Ji, L.L. and Fu, R. 1992). Some of these products of lipid peroxidation are toxic to various cells, including endothelial cells (Sasaguri et al., 1984). Lipid hydroperoxides decompose to produce aldehydes (e.g. malondialdehyde) and other products, including gaseous hydrocarbons such as ethane and pentane (Pryor, 1978 and Cohen, 1979). Their decomposition is catalyzed by transition metal ions and by haem compounds (Svingen et al., 1979; and Kohn and Kessel, 1979). Lipid hydroperoxides and some of their degradation products are highly cytotoxic: they cause extensive damage to enzymes and to membranes, producing a decrease in electrical resistance and

membrane fludity and eventual loss of membrane integrity (Gutteridge et al., 1979; Gardner, 1979; and Pauls and Thomas, 1980). Further, there is some evidence that malondialdehyde is a mutagen. Disruption of lysosomal membranes by lipid peroxidation can spill hydrolytic enzymes.

The peroxidation observed was probably initiated by traces of metal ions, especially iron, contaminating the reducing agents. Since Fe^{2+} is a good initiator of perxodation, these compounds probably serve to keep the iron in the reduced form so as to allow continuation of peroxidation (Wills, 1960 and Gutteridge et al., 1979). As a second protective mechanism, the chain reaction of lipid peroxidation can be effectively inhibited by abstraction of H from anothe donor (often called a "scavenger") to yield a donor radical that is relatively unreactive.

$$\text{Lipid-}O_2\text{+donor-H} \longrightarrow \text{Lipid -00H + donor}$$

It is well known that unsaturated fatty acids or lipids will undergo oxidation in the presence of oxygen. The progress of this reaction can be monitored in several

ways. As oxidation proceeds, it is possible to observe: (1) an increase in absorbance at 233 nm; (2) O_2 uptake; (3) an increase in the rate of lipid peroxidation present; and in some cases, (4) an increase in the amount of malondialdyhyde formed which is usually quantified by its highly coloured reaction product with thiobarbituric acid (TBA) test (Barber and Bernheim, 1969).

The increase in absorbance at 233 nm is attributed to the formation of conjugated diene systems in unsaturated lipids. Oxygen uptake results in the formation of peroxides whose concentration can be determined using an iodometric procedure. The TBA test for lipid peroxidation is a sensitive and widely used assay for malondialdyhyde formed during lipid peroxidation.

Lipid peroxidation is an autocatalytic free radical process (Pryor, 1978). Free radicals are short lived, highly reactive chemical species involved in a variety of functions, namely, oxidation of unsaturated fatty acids in cell membranes (lipid peroxidation), damage of DNA,

modulation of nucleotide cyclase activites and synthesis of prostaglandins and lipo-peroxides. These free radicals are usually produced in biological system during anti-microbial defense, through the action of mixed function monoxygenases, by various oxidative enzymes, such as xanthine oxidase and by auto-oxidation mediated by heavy metals and quinones (Proctor and Reynolds, 1984; Richter, 1988; Simic, et al., 1989). The H_2O_2 and other reactive O_2 species, if not scavenged efficiently, are known to give rise to potentially toxic intermediates, namely, hydroxy radical ($OH^{\bullet}$) and singlet ($O^{\bullet}_2$). These oxidants, in the presence of metal ions, result in the formation of lipid peroxides (Lai and Piette, 1978; Lai et al., 1979a).

Initiation of lipid peroxidation in a membrane or free fatty acid is due to the attack of any species that has sufficient reactivity to abstract a hydrogen atom. Since a hydrogen atom has only one electron, this leaves behind an unpaired electron on the carbon atom. The carbon radical in a polyunsaturated fatty acid tends to be stablized by a molecular rearrangement to produce a conjugated diene, which rapidly reacts with O_2 to give

hydroperoxy radical. Hydroperoxy radicals abstract hydrogen atoms from other lipid molecules and so continue the chain reaction of lipid peroxidation. The hydroperoxy radical combines with the hydrogen atom that it abstracts to give a lipid hydroperoxide (Barber and Bernheim, 1967). The classically accepted mechanism of free radical lipid peroxidation is outlined below.

[LH = fatty acid; LOOH = lipid hydroperoxides; $L^{\cdot}$ = lipid alkyl radical; LOO = lipid peroxy radicals]

Initiation

$LH + O_2 \longrightarrow$ Free radicals, $L^{\cdot}$ (i)

$LOOH \longrightarrow$ Free radicals, $L^{\cdot}$ (ii)

Propagation

$L^{\cdot} + O_2 \longrightarrow LOO^{\cdot}$

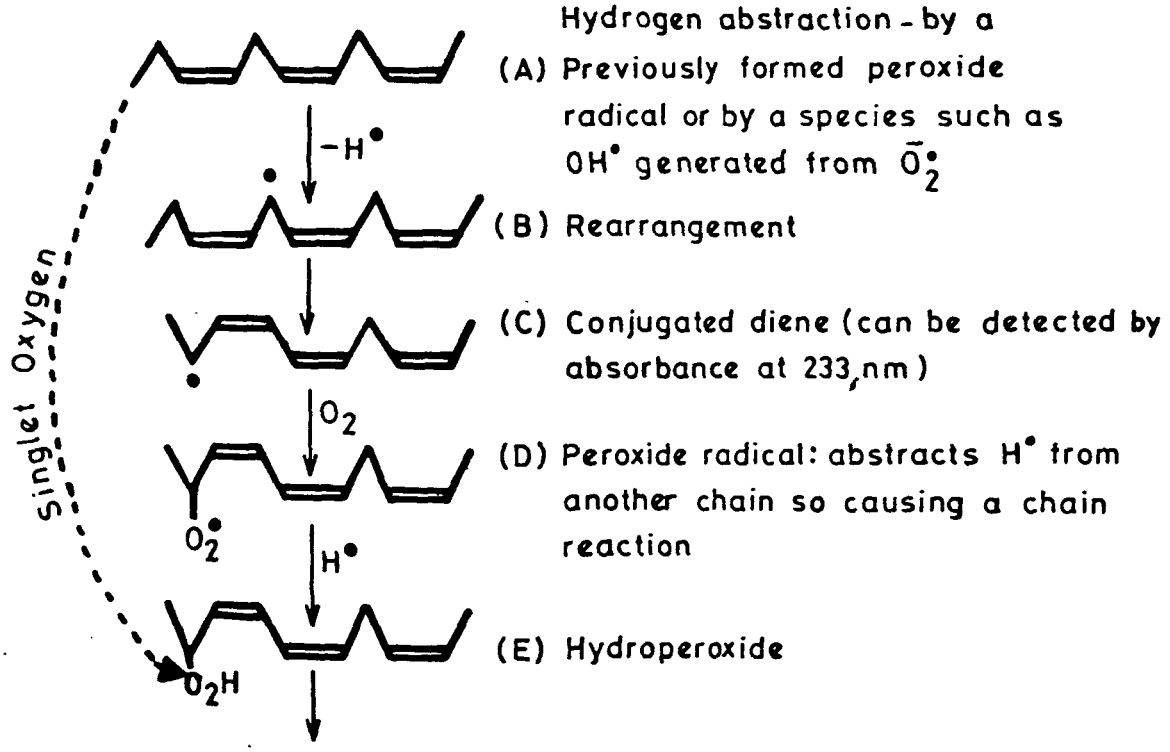
$LOO^{\cdot} + LH \longrightarrow LOOH + L^{\cdot}$

Termination

$LOO^{\cdot} + LOO^{\cdot} \longrightarrow LOOL + O$

$LOO^{\cdot} + L^{\cdot} \longrightarrow LOOL$

$L^{\cdot} + L^{\cdot} \longrightarrow LL$



Fragmentation products including (especially malondialdehyde)

Mechanism of peroxidation of polyunsaturated fatty acids.

Mechnism For Lipid Peroxidation: Dahle et al., (1962)

Lipid peroxidation is aimed to explain the following observations: —

the increase in absorbance at 233 nm in the early stage of the oxidation, the appearance of lipid peroxides as intermediate in the reaction, the inhibiting effect of chain breaking antioxidants on the reaction, and the more facile production of malondialdehyde as quantified by the TBA test from linolenic or arachidonic acids when compared to linoleic acid. The steps in the oxidation of a diene and a triene fatty acid system are illustrated in Fig.A. This free radical chain reaction is initiated when some unidentified free radical abstracts a methylene H atom from the unsaturated fatty acids. The resulting free radical is stablized by resonance, with several of the resonance forms adopting a conjugated diene system. Oxygen adds to the lipid free radical producing a hydroperoxy radical which may abstract a hydrogen atom from another unsaturated fatty acid (thereby propagating the chain reaction) to form a

lipidperoxide. In this mechanism, malondialdehyde is produced by the triene but not the diene system because only with the former is it possible to obtain a hydroperoxy radical with a double bonds to the peroxy radical. It is possible to obtain the cyclic peroxide II in triene systems which is the non-volatile precursor of malondialdehyde. Pryor, et al., (1976) have criticized this mechanism because of the assumption that only methylene hydrogen atoms can be abstracted in the initial step. If hydrogen abstraction can also occur at alkylic positions at the ends of the alkene systems then linoleic acid can also produce the cyclic peroxide II. Since both linoleic and linolenic acids can form II, then Dahle's mechanism does not explain the difference in the state of malondialdehyde formation from linoleic and linolenic acids. Pryor, et al., (1976) also suggest that the fatty acid free radical I abstracts a hydrogen atom internally. Triene systems produce a more suitable bicyclic free radical III than diene systems since they are stablized by the third double bond.

The endoperoxide is the non-volatile malondialdehyde precursor in Pryor's mechanism. The

endoperoxide has a structure related to those of the endoperoxides produced in bio synthetic sequence leading to prostaglandins and have also shown that many cyclic peroxides produced during the oxidation of unsaturated fatty acids give a positive TBA test (Pryor et al., 1976). In fact, of the 5 cyclic peroxides only the one with dioxygen functionally on a tertiary carbon failed to give a positive TBA test. Hence, there is probably more than one malondialdehyde precursor. However, the activation of O_2 is not the only mechanism of activation of lipid peroxidation. But it is also the process of a chain reaction by its nature, and the influence on the proceeding of the reaction may be the key mechanism of lipid peroxidation regulating in the cell. Studies from our laboratory (IBRC) have shown that the lipid peroxidation 'in vivo' is of basic importance in aging, in damage to cells by toxic gases, heavy metals and organophosphate pesticide neurotoxicity (Gupta and Hasan, 1988; Haider and Hasan, 1984; Haider et al ., 1981; Hasan and Ali, 1981; Bano and Hasan, 1989; Vadhva and Hasan, 1986; and Naqi et al., 1988; Myshkin et al., 1992; Milan and Zuzana, 1996 and Masayuki, et al. 1996).

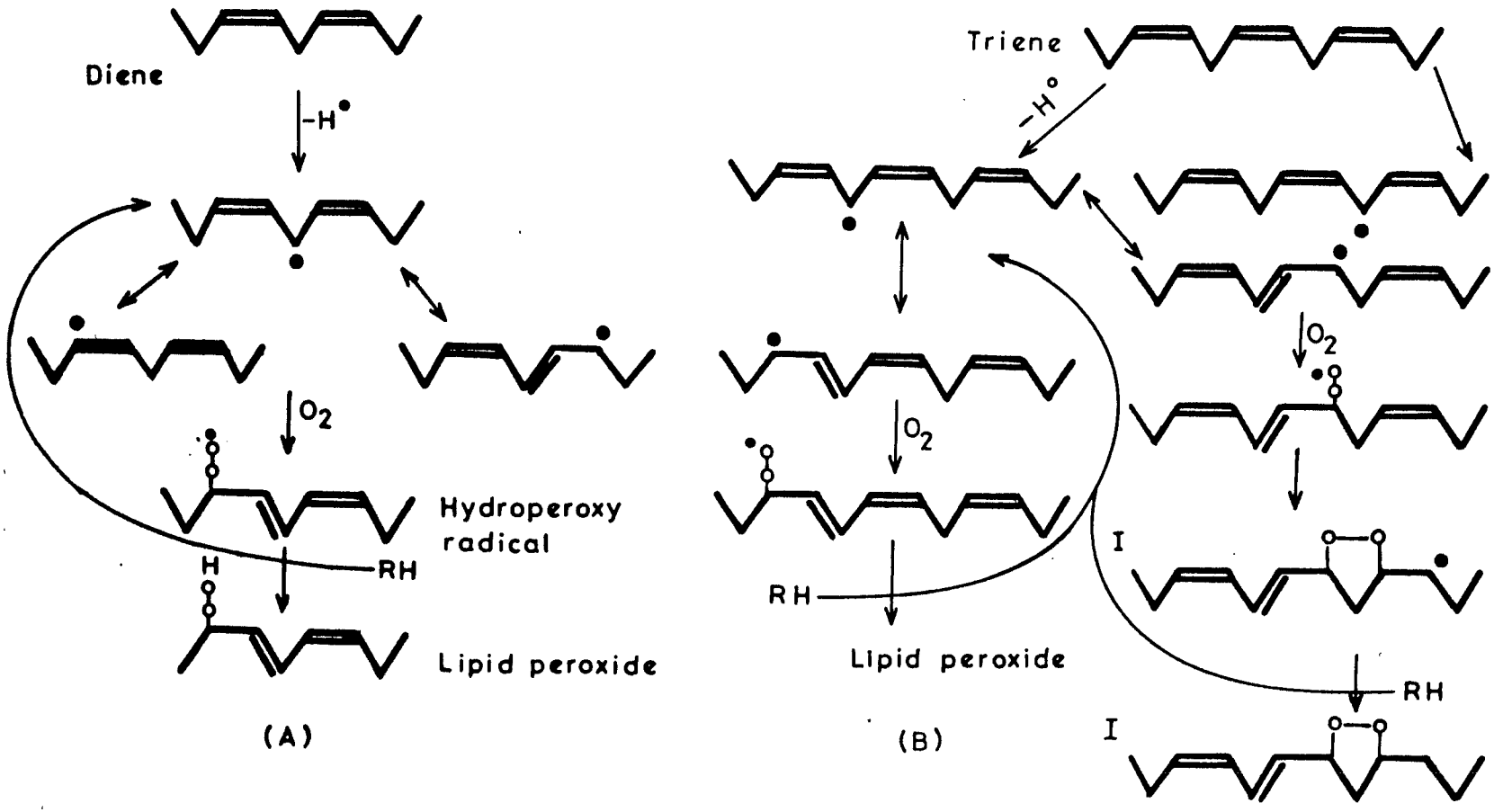


Fig. A.

Oxidation of a diene (A) and a triene (B) fatty acid system adapted from the mechanism proposed by Dahle et al. (1962). I the triene mechanism the oxidation of only one of the fatty acid radicals initially produced by hydrogen abstraction is shown. The oxidation of the other free radical is similar to the mechanism presented. RH, a polyunsaturated fatty acid.

The problem of controlling mechanisms of lipid peroxidation in the cell has become more and more complex.

To date, the effect of AlP pesticides on brain and the rate of lipid peroxidation are limited particularly the effect of AlP on the lipid peroxidation is not known. Therefore, it would be of particular interest to investigate the rate of lipid peroxidation in discreate brain areas after the administration of AlP solution.

2.21 Sulfhydryl Groups (Thiol Groups; SH):

They play a pivotal role in many important enzymes by acting as active enzymatic sites (Hoch and Vallee, 1959). In principle, any enzyme bearing an accessible thiol essential for activity is capable of forming protein mixed disulfides or intramolecular disulfides can increase or decrease catalytic activity and examples of both are known. Furthermore, the extent of enzyme-s- thiolation would depend on the thioldisulfide redox potential as well as the nature of the small

disulfide and the microenvironment around the accessible protein thiol. These parameters are at least potentially capable of conforming to the specificity required for a biological control mechanism through signals transmitted by changes in the thiol - disulfide redox potential as a function of different metabolic states.

Sylfhydryl groups derived from the side chain of cysteine residues, occur in a number of enzymes. Sulfhydryl (-SH) groups and disulfide (- SS groups) bond of cysteine are highly reactive and apparently involved in the maintenance of the conformation and biological activity of certain proteins. As the receptors are protein in nature, the reagents which modify - SH groups may influence the interaction of neurotransmitters with their recognition sites (Sobrino and Del Castillo, 1972).

Sulfhydryl groups play an important role in GST induced detoxification against electrophilic xenobiotics and toxicants by conjugating with such compounds and thus neutralizing their electrophlic sites (Habig et al., 1974).

Glutathione has been considered to function as biological antioxidant. It plays a pivotal role in the destruction of free radicals as well as inorganic and organic peroxides (Sohal et al, 1984; Ji, L.L. and Fu. R. 1992). GSH is a naturally occuring and widely distributed tripeptide. It consists of glycine, cysteine and glutamic acid moities (Allen and Balin, 1989). It is the major non protein thiol compound present in cells in concentrations which range between 0.1 and 10 mM (Kosower, 1976a). It is synthesized intracellulary by the consecutive actions of glutamyl cysteine synthase and GSH synthase. Its concentration is dependent on metabolic rate and the level of oxidative stress (Allen et al, 1985 a). It has been implicated in a wide variety of biological functions, such as the maintenance of cell membranes, destruction of metabolic peroxides and free radicals, detoxification of foreign compounds, removal of H_2O_2 , maintenance of thiol group of enzymes and proteins, control of redox status, disulfide exchange reactions and transport of amino acids and peptides across membranes (Hazeltona and Lang 1980 and; Ziegler, 1985).

Katoh et al. (1989) observed an enhanced level of lipid peroxides associated with the GSH depletion. The role of GSH in peroxidation is evidenced by the inhibition of oxidative stress induced by different compounds such as ascorbate, NADPH-$BrCCl_3$ and NADPH-Fe^{2+} (Tampo and Yanaha. 1990). The GSH was observed to protect rats from toxic species engendered by hyperoxia (Van et al, 1985). White et al. (1988) observed that GSH redox cycle increases survival and detoxification of H_2O_2 in hypoxia pre-exposed rats and contributes to tolerance to hyperoxia. Gupta et al. (1986) observed a significant increase in GSH level with antioxidant in mice. Rotruck et al. (1972) reported that Se-glucose - GSH system plays a dual role in the preservation of the integrity of the cell membrane and of haemoglobin aginst haemolysis and oxidative damage.

In 1970, Tappel had suggested that deficiency of total and free sulfhydryl groups may lead to deficient degradation of lipid peroxides to hydroxy acids, causing accumulation of peroxides in various regions of the brain.

The concentration of oxidized glutathione or glutathione disulfide (GSSG) reported for various tissues range between 4 and 50 mM (Tietze, 1969). A slight increase in the concentration of GSSG even in the presence of a large excess of GSH, has effects of potential physiological importance (Kosower and Kosower 1974 a). One potent physiological function of the activity of GSSG in inhibiting protein synthesis might be as a control mechanism. If the concentration of GSSG within the cell rises above a certain level, initiation factors are converted into an inactive form and the total rate of protein synthesis decreases. According to Zehavi-Willner et al. (1971) alterations in the GSH/GSSG ratio may also be related to the enhanced rate of protein synthesis, GSH/GSSG ratio is not effected by exercise or by malatonin treatment (Masayuki, et al. 1996).

2.22 Superoxide Dismutase (SOD):

Oxygen is utilized by all aerobic organisms that must have some mechanism by which they can minimize toxicity. One mechanism is the production of superoxide

radical and its dismutation reaction, catalyzed by the enzyme superoxide dismutase (Harman, 1956; 1971). The superoxide anion is a free radical formed by one electron transfer to oxygen by so many spontaneous and enzymatic oxidations (Mishra & Fridovich 1972; Marklund & Marklund, 1974).

$$O_2 + e^- \longrightarrow O_2^{\bullet}$$

Superoxide dismutase (SOD) catalyzes the dismutation between two moles of superoxide anion to yield one mole of oxidized product (oxygen) and one mole of reduced product (Hydrogen peroxide) (Klug, et al. 1972).

$$O_2^{\bullet} + O_2^{\bullet} + 2H^+ \longrightarrow O_2 + H_2O_2$$

This is analogous to the dismutation of hydrogen peroxide to oxygen and water catalyzed by catalase enzyme. Ordinarily, electrostatic repulsion between two molecules of superoxide anion. Their approach to one another; SOD overcomes the barrier greatly increases the dismutation rate (Fridovich, 1976; 1978).

SOD appears to protect against the toxic effects of the $O_2^{\cdot}$ free radical and thus provides a mechanism

whereby an organism can avoid possible deleterious effects of this radical or other free radicals which might be produced by its further reaction with cellular components (Fridovich. 1975; Mc Cord et al, 1971). Superoxide arises naturally in some enzymatic reactions (Fridovich, 1978) such as those catalyzed by xanthine oxidase, dihydro-oratic acid oxidase, aldehyde oxidase, trypotphan dioxygenase, or during autoxidation of tissue constituents such as reduced flavins of ascorbate or more dramatically during the rapid spontaneous auto-oxidation of certain neuronal toxins such as 6-hydroxydopamine or 6-aminodopamine (Cohen and Heikilla, 1974). Superoxide radical at neutral pH can act either as a weak oxidizing agent, e.g. with catecholamines, or as a strong reducing agent, e.g. with cytochrome C.

The SOD enzyme was first discovered in 1969 by McCord and Fridovich and serveral forms have been identified. They identified the enzymatic activity associated with erythrocuprein, a copper-zinc protein of erythrocytes. The copper is associated with enzymatic activity, whereas the zinc is structural. Similarly, SOD activity is associated with a family of copper-zinc

proteins, cerebrocuprein in brain (Fried, 1979) and hepatocuprein of liver. In mammalian tissues, a second form exists in which manage the prosthetic group (Fridovich, 1976). SOD is localized to mitochondria, whereas the Cu-Zu SOD is cytoplasmic. However, this distribution does not hold in other species.

Regional distribution in the rat showed a relatively homogenous distribution in brain about a twofold range from the highest area (medulla oblongata) to the lowest area (cortex), also subscellular disribution studies in the rat showed the highest level in the cytoplasm while myelin has very low levels. Thomas et al., (1976). indicated that very high levels of activity are present in liver, while the adrenals, kidney and red blood cells have intermediate activity, and lower activities were found in most other tissues including brain (Fried and Mandel (1975).

The principal causes of peroxidative damage has been implicated (Barber and Berheim, 1967; Hougarrd, 1968). Such damage is at least partially associated with the free radicals. The reduction in SOD activity as a

function of age could result in an impaired protection against the toxic effects of O_2 and thus might lead to serve cellular damage (Kellogg and Fridovich, 1976; Vanella et al, 1982, Tayarami et al, 1989). No report, however, is available todate on the effect of AlP toxicity on the SOD activity.

2.23 Glutathione Reductase (GSSG-R or GR):

Glutathione reductase, a heat labile enzyme catalyzes irreversible conversion of GSSG to GSH and accounts for very high GSH:GSSG ratio in the cells. The reaction takes place according to the following equation.

$$GSSG + NADPH \xrightarrow[H^+]{GSSG\text{-}R} 2GSH + NADP^+$$

GR is reckoned to be as ubiquitous as glutathione and has been studied in various tissues (Ray and Prescott, 1975; Ormstad et al., 1979). This enzyme is isolated from human platelets (Moroff and Kosow, 1978), leucocytes (Ogus and Tezcan, 1981) and erythrocytes (Chang et al., 1978). The primary and unambiguous role of glutahione reductase is of course, to regenerate reduced GSH that has been oxidized (i) non-specifically

by oxygen radicals or peroxides, (ii) enzymatically through the GSH peroxidase reaction, (iii) spontaneously or enzymatically by means of thiol-disulfide exchange reactions or (iv) possible by other redox reactions.

Glutathione reducatse has been illustrated to be an inducible enzyme when rat liver cells were treated with various compounds which suggests that GR is of great importance for the protection of cells against toxic agents (Carlberg et al., 1981). The destruction of GSHPx, GSSG-R and SOD activities was found to be the underlying cause of free radical damage caused by reperfusion injury of rat kidney (Okajima, 1990). Benzi et al. (1989) measured the activities of enzymes related to the anti-oxidant system in different regions of brain of rats. In general, both SOD and GR tended to decline during the last half of life. The GSSG-R activity was maximum at 25 days after birth in rats, afterwards the activity decreased continuously in adults but again increased during the developing period, especially in female rats (Santa and Machado, 1986). Stohs et al. (1984) reported that GR activity and GSH content were higher in erythrocytes from mature and middle aged

humans followed by a considerable decline contributing to senescene and increased susceptibility to carcinogenesis and drugs.

2.24 Glutathione Peroxidase (GSHPx; Gluatahione: H_2O_2 Oxireductase):

GSHPx to be a peroxidase in red blood cells and in a variety of tissues (Mills, 1957). The enzyme would be detoxify lipid peroxides by converting the peroxides to their corresponding monohydroxy unsaturated fatty acids (Little and O'Brien, 1968). The reduction takes place at the expense of donor substrate, GSH, which is hydrogen donor to reduce hydroperoxides to the corresponding alcohols.

$$2GSH + ROOH \longrightarrow ROH + H_2O + GSSG$$

where R = CH_3 or any alkyl group.

These acceptor substrates comprise a variety of biochemically important compounds, such as unsaturated lipids, steroids, nucleic acids (Flone and Gunzler, 1974) and prostaglandins (Nugteren and Nazelhof, 1973).

The enzyme occurs in two forms (i) selenium dependent GSHPx (it catalyzes the reduction of all

hydroperoxides including H_2O_2) and (ii) selenium independent GSHPx (it catalyzes the break-down of only organic hydroperoxides).

The presence of peroxidase in various tissues and the ability of the enzyme to metabolize peroxides of any structure at similar rates has led to the suggestion that GSHPx is the main product within the mammalian cell from peroxidative damage (Chow and Tappel, 1972; Chow et al, 1973). Multiple cellular functions are regulated by GSHPx such as cell division (Kosower and Kosower, 1974a) pentose phosphate shunt (Eggleston and Krebs, 1974) and mitochondrial oxidation of 2-oxoacids (Sies and Moss, 1978). The role of the GSHPx in maintaining the integrity of the erythrocytes membrane has been extensively studied (Beutler, 1972).

Owing to the high concentration of polysaturated fatty acids to peroxidative damage GSHPx could provide a mechanism to protect brain tissue against this type of damage. A limited number of studies have been performed with rat brain. A species comparison of levels of GSHPx in the cytosolic fraction was carried out of brain (Demrchine et al.,

1974). The study was performed with unperfused brains may be object to contamination by erythrocytes which contain much higher levels of enzyme.

The increased conentration of GSHPx in growing mouse kidney was reported (Su et al., 1979 and Barlo-Walden, 1995), while blood GSHPx showed an increase only in vitamin E supplemented animals (Pieri, et al., 1994). Other tissues, like lung, liver, uterus and spleen, do not show any increase in the enzyme activity. The GSHPx and catalase increased with age and decreased their highest values by adulthood or senscence respectively in the subendocardial region of heart (Simonetti et al., 1990). Hazelton and Lang (1985) observed GSHPx and SOD showed a lower constant specific activity during the development with a post-natal increase upto adult age of isolated hepatocytes. GSHPx activity was inhibited by H_2O_2 (Ochi, 1990b) but the H_2O_2 cumene hydroperoxide and t-butyl hydroperoxide as substrates increased GSHPx activity more than 1.5 fold over the period of 1-12 months and remained high in old rats (Lemeshko et al., 1985). Following vigorous exercise, plasma lipid peroxide concentration was

increased and GHSPx activity significantly reduced (Masayuki, et al. 1996).

2.25 Glutathione-S- Transferase (GST):

It is a non selenium dependent glutathione peroxidase (Sies it al., 1979). GST was first identified in rat liver cytosol (Both et al., 1961; Coombs and Stakelum, 1961). The enzyme was subsequently named glutathione-s-aryl transferase. Later on, several other GSTs were demonstrated depending upon the substrate specificity. GSTs are of three types-

i) Glutathione-s-alkyl transferase as a catalyst in numerous reactions in which glutathione participates as a nucleophile (Johbson, 1966);

ii) Glutathione-s-epoxide transferase, active towards the conjugation of active epoxides with glutathione (Boyland and Williams, 1965).

iii) Glutathione-s-alkene transferase, catalyzing the conjugation of unsaturated compounds with glutathione (Covalent linkage).

The concentration of GST is, in general, high in mammals (upto 10% of cytosolic proteins in some organs). In other species (Shark) the level of activity is quite low (Sugiyama et al., 1981). The GSTs are a famjly of multifunctional proteins that function both as important enzymes of detoxification and intracellular binding protiens (Boyer, 1989). As enzymes, they catalyze the reaction between nucleophil reduced GSH and large number of electrophilic compounds such as polycyclic aromatic hydrocarons, aromatic amines, azodyes, alkylating agents, carcinogens and neurotoxins (Habing et al., 1974; Smith et al., 1977 & Chasseaud, 1979). Additionally, a number of endogeneous compounds, including prostaglandins, leucotrienes, organic hydroperoxides (including lipid hydroperoxides and products of lipid peroxidation) and steroids act as substrate for GST (Jakboy, 1978; kaplowitz, 1980). GST catalyzed reactions produce two types of products (Douglas, 1987).

i) a stable gultathione conjugate is formed by the nucleophilic attack of GSH on an electrophilic centre. These types of reactions occur with

substrates such as epoxides (metabolites of benzo)

$$R\text{-}X + GSH \xrightarrow[\text{acetaminophen}]{P_{450}} R\text{-}SG + XH \quad (i)$$

Where X is a leaving group.

ii) A reduced substrate and glutathione disulfide (GSSG) are formed. In this reaction, an unstable R-GS intermediate is the enzymatic product (Eq. ii) which is attached nonenzymatically by a second molecule of GSH, yielding the final product and GSG (Eq.iii). Examples of substrates for this second type of reaction are organic nitrates and organic hydroperoxides.

$$R\text{-}X + GSH \longrightarrow R\text{-}GS + XH \quad (ii).$$

$$R\text{-}GS + GSH \longrightarrow RH + GSSG \quad (iii)$$

Todate no information is available in the literature on the possibile alterations in GST activity in the different regions of rat brain following AlP intoxication. In the present work, it is planned to study the GST activity to evaluate the neurotoxicity of AlP.

2.26 Monomine Oxidase (MAO):

In 1972, Costa and Sandler discovered MAO. It is a flavin-containing enzyme located on the outer membrane of the mitochondria. Oxidative deamination of primary monoamines by MAO produces NH_3, aldehydes and H_2O_2 agents with established or potential toxicity (Cooper et al., 1978; Benedetti and Dostert, 1989).

$$RCH_2CH_2NH_2 + O_2 + H_2O \xrightarrow{MAO} RCH_2CHO + H_2O_2 + NH_3$$

MAO is one of the major mammalian neuronal enzymes. It is active in both neurons and gilal cells in the brain . MAO plays a strategic role in inactivating catecholamines that are free within the nerve terminals and not protected by the storage vesicles (Coyle and Snyder, 1981). When monoamines leak from the synaptic vesicles, MAO acts within the nerve fibre itself. The enzyme serves to oxidize some of the 5-HT, DA and NE after their release into the synaptic space in the nervous system, thus terminating their action. The concept of two functionally distinct forms of MAO has gained wide acceptance (Houslay et al., 1976 and Leung et al., 1981).

The MAO-A deaminates neurotransmitter amines such as 5-hydroxytryptamine (5-HT) and noradernaline (NA) and is inhibited sepcifically by glergyline [N-methyl-N-propargyl- 3 (2,4, dichlorophenoxyl) propylamine]. Whereas type B oxidizes benzylamine and β phenylethylamine and is preferentially inhibited by deprenyl phenylisopropylmethylpropinylamine (Tipton and Dellacorte, 1979). Both forms deaminate substrates such as tyramine and tryptamine (Houslay et al., 1976).

The MAO-B activity increases in human brain in ageing (Robinson et al., 1972), while the MAO-A activity either increases (Shih, 1979) or remains unchanged (Fowler et al., 1980). In old rats MAO-A activity was decreased significantly in all the regions studied except in the cerebellum, where it was unchanged. On the other hand, MAO-B activity increased in all the areas studied except in the brain stem, where it decreased (Leung et al., 1981: Danh et al., 1984). This suggests that the mechanisms which alter the activities of the two forms are not related with each other. Any change in the usual amine concentration will disturb

their activities and result in convulsive seizures (Killian and Frey, 1973). Hence, it is likely that AlP also influences the monoamine concentration in the brain and might also be acting through this mechanism in producing central toxic effects.

2.27 Nucleic Acids (DNA and RNA):

Nucleic acids play important role in protein synthesis. In the brain the storage and transmission of genetic information as well as translation of this information leading to the systhesis of cellular proteins (White et al., 1978).

The tissue components such as average cell densities, dry weight/average cell and total number of cells in each brain area understanding with the help of DNA (May and Grenell, 1959). Generally cells of brain are diploid and contain a fixed quantity of DNA per cell (Heller and Elliot, 1954). The amount of DNA in white matter approximately equals that in the cortex, and regional differences in the amount of brain DNA are relatively small (Elliot and Heller, 1957). However, only cerebellum has exceptionally high amounts of DNA (May and Grenell, 1959).

The central role of DNA is information transfer between generations of somatic cells. Burger (1957) found parallel changes of DNA in growing brain. The low point for DNA is in the third decade, the time at which the brain reaches its greatest weight. As the dry weight of the brain decreases steadily from the third decade of life through the ninth, it is important to estimate if this apparent increase is a real one in terms of the total amount of DNA present in the brain. To answer this question, the weight in grams of dry matter on the basis of the average size brain for each decade of life was calculated. The data suggested that in the old brain, although there was loss of protein and lipid, there might be at the same time an increase in DNA which might be imputed in part to a proliferation of the glial elements. The increase in DNA in old age is due to two factors: an increase in pyknosis of the neurons and growth of glial elements (Burger, 1957). The decrease in DNA in the cytoplasm of brain cells from old rats (Hyden, 1955) and break down of DNA in the presence of deoxiribonuclease enzyme. From our laboratory, Tayyaba et al. (1981) reported that there was a remarkable decrease in the

DNA level in all the brain regions studied after 'metasystox' toxicosis.

A knowledge of RNA is very helpful in the study of the rate of protein synthesis and also in understanding the functional status of the nervous tissue (Bergen et al., 1974; Vijaya Kumar, 1987). The RNA amount in gray matter is usually higher than in white matter (Mihailovic et al., 1958). The nucleolus and in the Nissl substance of the cytoplasm of nerve cells have more RNA concentration (Landstrom et al., 1941). The RNA concentration has also shown variations within different brain regions, the highest concentration being in cerebellum, hypothalamus and cerebral cortex and the lowest in medulla (May and Grenell, 1959). The break down of RNA takes place with the help of ribonuclease enzyme.

RNA and protein synthesis may be involved in the accrual of sensory information in the brain, thus indicating a possible approach to elucidation of brain function on a molecular basis (Hyden, 1964). Edstron (1956) and Edstron and Pigon (1958) have reported that there is a proportionality between RNA content and the

surface area of the cell body. Hyden (1964) has reported that the content of RNA neurons vary over a wide range. Increased RNA concentration due to metasystox neurotoxicity in cerebellum, brain stem, and spinal cord in the rat brain have been reported (Tayyaba et al., 1981 Sastry and Siddiqui, 1984). The available literature indicates that the effects of OP compounds on brain nucleic acids is still inadequately understood. As the brain regions show remarkable heterogeneity in nucleic acid contents, it is reasonable to investigate the neurotoxic effects of OP compounds and other pesticides on discrete brain areas. The present work deals with the effect of AlP on nucleic acids level in various regions of rat CNS.

2.28. Protein:

Proteins specific to the nervous system are of interest because they underline the developmental specialization and differentiation of the system's cells. These proteins are usually assayed in terms of their biological activity, for example, as enzymes or receptors for specific ligands or, more generally, as antigens. These properties are also frequently employed as aids in

the isolation and purification of the proteins. Many of the important proteins of nerve tissues, including entities at the synapse, are glycoproteins, that is, they contain oligosaccharide side chains attached to selected aspartate, and perhaps serine and threonine, residues of their polypeptide chains (Mahler, 1978).

Protein, one of the many important biochemical components in the vertebrate brain, constitutes 40% of the dry weight (McLlwain & Bachalard, 1971). The changes in the neuronal activity are accompanied by measurable changes in macromolecules like protein in brain cells. It has also been reported that the increased neuronal activity decreases or inhibits the synthesis of proteins (Hyden and Lange, 1972). The specific neuronal functions such as condution of action potentials, and synaptic transmission are extensively mediated by protein (Block, 1978). Recent evidences suggest the role of glycoproteins in a number of specific cell-cell interactions, including intercellular adhesion and the mechanisms governing neural histogenesis, regional brain differentiation and the specificity of neuronal associations (Margolis et al., 1975).

Takehara (1956 and 1957) mentioned the existence of a species-specific fraction and an organospecific fraction in the brain proteins. Also Caspara and Fiel (1963) described a brain specific antigen. The S-100 protein (which is heterogeneous) is distributed in all parts of the nervous system, both peripherally and centrally. It is probably a neuronal protein composing no part of the myelin sheath structure (Moore, 1965). Proteins in the brain are in a dynamic state. Synthesis and catabolism have been intensively studied by Lajtha (1961).

Proteins are both implements and modulators of the autocatalytic and heterocatalytic system charged with genetic continuity and its expression, that is, as components of the replicative, transcriptional, and translational apparatus. Proteins destined for intracellular use, including peripheral membrane proteins and proteins residing on the cytoplasmic aspect of plasma membranes, are synthesized by polysomal arrays in the cytosol, that is, unattached to membranes. Recent studies have disclosed that many proteins are subject to the post translational modification by

controlled proteolysis of defined segments of the newly synthesized polypeptide chain (Lodish, 1976). It has been verified for protein synthesis in Aplysia, is discrete neurons and neuronal clusters with defined function (Berry, 1976 and Loh et al., 1977).

It has been evidenced that many environmental and nutritional factors may bring about the changes in the proteins McLlwain and Bachelard, 1971). The decrement in the protein concentration in various regions of CNS has been observed in the rats treated with different doses of metasystox (Tayabba et al., 1981).

Brain, in general, has high rate of metabolic activities. It needs more proteins for expected high rate of protein turn over. This view is well correlated with the presence of large amount of cytoplasmic ribosomes, which gives large number of sites for protein synthesis (McLlwain and Bachelard, 1971). Any change in the protein concentration may influence the metabolic rate of the tissue. It requires rapid synthesis and renewal of protein. To analyse this view, in the present work, an attempt has been made to study the changes in the protein content of rat brain due to AlP treatment.

2.29 Acetylcholinesterase (AChE; Acetylcholinehydrolase):

AChE is one example of an enzyme that funtions extracellularly; it is localized at functionally specialised parts of plasma membranes, such as the end-plate region of skeletal muscle. This enzyme has an attachment segment that resembles collagen in structure and composition (Lwebuga-Mukasa et al., 1976). AChE of skeletal muscle is located in neuromuscular junction, were it hydrolyzes the ACh released from the nerve terminal. Three lines of evidence indicate that AChE is associated with the basal lamina of muscle rather than being an integral part of the postsynaptic plasma membrane. First, mild chemical treatments release AChE from skeletal muscle or the electric organs of Torpedo. Tissues treated by mild enzyme hydrolysis (Massoulie et al., 1970), or extracted with high ionic strength solutions (Hall, 1972) release AChE. Second, the chemical structures are attached to a filamentous tail that resembles collagen fibrils present in the basal lamina (Taylor et al., 1977).

AChE of brain behaves as if it were assessible to quaternary substrates and inhibitors (Koelle and

Coauthor, 1963). They supposed that this fraction of the functional AChE is outward facing, and the reserve AChE is inward facing and in transit. The ability of peripheral cholinergically innervated tissues to form surplus ACh (Collier and Katz, 1971) in the presence of an anticholinesterase suggests that at least a small part of the enzyme transported by peripheral axons is still in the reserve orientation as it nears the synapses; cerebral cortex, which seems to have less of that ability, may posses only functional AChE. Most tissues contain several forms of AChE (Ulus et al., 1978, Bon et al; 1979; Fasbraey, et al., 1990: and Shish, et al., 1993). These are catalytically identical glycoproteins, but they differ in molecular weight, ease of extraction and physical properties.

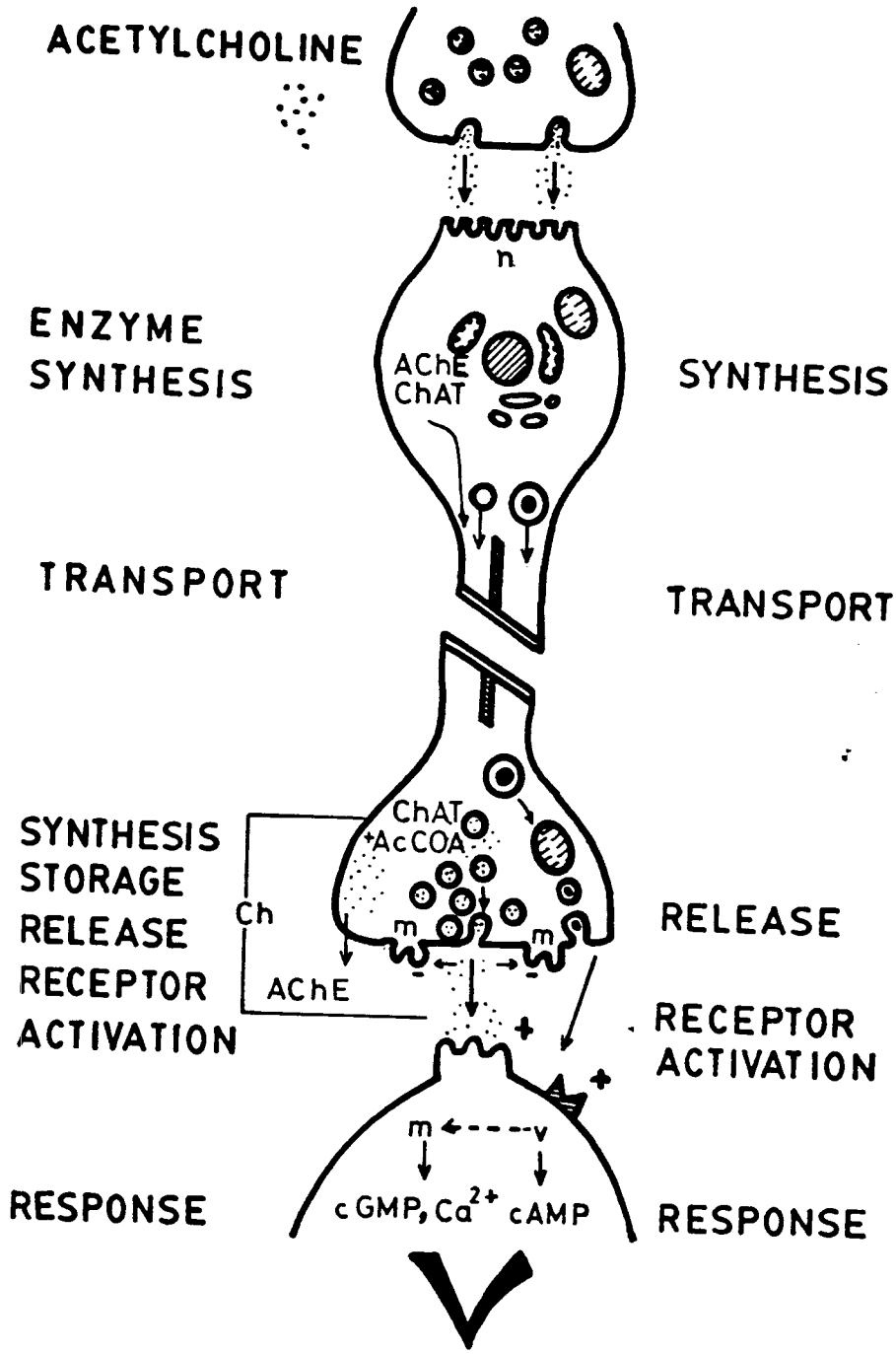
The most conspicuous feature of all organophosphorus compounds is their structural complementarity with the target enzyme molecule, ChE. In essence, organophosphorus compounds mimic the gross molecular shape of the natural substrate of ChE, ACh. ChE is perhaps one of the most studied enzymes in biological systems. The organophosphorus pesticides

mimic the natural substrate, ACh, by binding itself to the esteratic site of AChE, resulting in the phosphorylation of the enzyme which is inacitve (Cremlyn, 1978). Parathion toxicity in rats showed reduced ChE activity (Du Bois et al., 1949). Subsequent researches have also shown that most of the OP's inhibit AChE in the brain of vertebrates after crossing the blood brain barrier (Emsley et al, 1976). Malathion also inhibited the activity of AChE in the rat brain (Paul et al., 1979). The organophosphorus compound diazinon inhibit ChE activity in the CNS and other parts of the body and induce hyperglycaemia (Dybing and Sognen, 1958 and Weiss et al., 1964) and increase the brain level of ACh (Kar and Matin, 1971), resulting in stimulatory effects, tremors and convulsions. The inactivation of AChE as result of OP poisoning results in the accumulation of ACh at nerve endings (Coppage et al, 1975; Cremlyn, 1978; Hall and Kolbe, 1980; and Fasbriey, et al. 1990). Inhibition of this enzyme by OP's is a result of firm binding of phosphate radicals of the OP's to the active sites of the enzyme (Johnson, 1976). This prevents the smooth transmission of nerve impulses across the synaptic cleft (Murphy, 1980), causing

restlessness, necrosis, tremor, ataxia, convulsions and depression of respiratory centres (Murphy, 1980). It has been demonstrated by 'in vitro' studies that the enzyme inhibited by dimethyl-p-nitrophenyl phosphate is more unstable than that inhibited by the diethyl analogue (Aldridge, 1971).

Anticholinesterase poisoning, if not too severe, can be relieved by treatment with atropine, supplemented by a suitable oxime (RCHNOH), if the poisoning is the result of a long-acting organophosphorus inhibitor. The oxime combines chemically with the phosphorus atom of inhibitor and so reactivates the enzyme. It was previously reported that diacetylmonoxime (DAM) readily crossed the blood brain barrier and was more effective than other oximes in reactivating the ChE in the brain (Holmstedt, 1959).

Krupka and Laidler (1961) were especially concerned with the structure of the active centre and the kinetics of enzyme action and inhibition. Their results provide good evidence on the active site of acetylcholinesterase and its mode of action. Investigations on the effect of OP pesticides on brain of



Schematic illustration of major characteristics of synthesis, release etc. for a postganglionic ACh containing neuron. ACh = dots, n = nicotinic receptors, AChE = acetylcholinesterase, ChAT choline acetyltransferase, Ch = choline, AcCoA = acetyl coenzyme A, m = muscarinic receptor, — = inhibitor of release, + = stimulation of receptor.

both target and non-target animals are abundant but literature on changes in AChE activity due to sublethal concentration are scanty, particularly in rats. Hence, an attempt have been made to study the extent of AlP neurotoxicity and protective effect of vitamin E against AlP in various regions of rat CNS.

2.30 Aims and Objectives of the Present Study:

Review of literature on Aluminium phosphide indicates that most of the investigations on brain focussed less attention to its regional heterogeneity. Since each region has its own function and biochemical constituents, it is now realised that more enlightment should come from the smaller divisions (Norton, 1980). Hence, in the present work, the rat brain has been divided into different regions i.e., cerebral hemisphere, (cerebrum) cerebellum, brain stem, and spinal cord to study the toxic effect of AlP.

AlP commercially known as Quickphos(R) (Celphos), is one of many pesticides that is of wide use as a fumigant rodenticide in India. The literature on the effect of AlP on rat CNS is meagre and insufficient to understand the neurotoxicity of this pesticide on non-

target animals such as rat. Hence, AlP has been selected for the present study, and vitamin E was chosen as antioxidant. The main objectives of the present investigations are as follows :

1. Open Field Behaviour (OFB) study was done on different parameters: i.e. Ambulation, preening and rearing.

2. To evaluate the quantitative effect of phosphine on the various neurochemical parameters in different regions of CNS. The following parameters were studied :

i. **Cellular components :** Total lipids & cholesterol.

ii. **Free radical substances :** Lipid peroxides, lipid hydroperoxides.

iii. **Antioxidant substance :** Total -SH, Free -SH (Reduced glutathione), oxidized glutatione (GSSG).

iv. **Antioxidant enzymes :** Super oxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GSHPx) and glutathione-S-transferase (GST).

v. **Catacholaminergic :** Monoamine oxidase (MAO).

vi. **Sub Cellular components :** Nucleic acids (DNA) and RNA) and protein.

vii. **Cholenergic system :** Level of AChE activity.

2. To observe the possible protective effect of antioxidant vitamin E on various neurochemical parameters. (i) LPO and LHPO (ii) GSH, GSSG (iii) SOD, GR, GSHPx MAO and GST.

2.31 Lacunae in Knowledge

A critical literature survey revealed that :

i. Brain lipids are essential components of all cellular structures and the level of lipids is altered during chemical stress. Regional studies of lipids in the different brain parts are relatively limited in number. The effect of AlP intoxication on lipids of the various regions of the CNS is not clear.

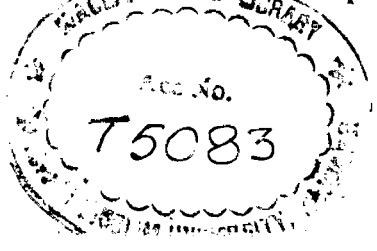
ii. Lipid peroxidation is one of the major causes of pesticide toxicosis. It is a free radical mediated chain reaction. During chemical intoxication oxygen species are readily generated. No study could be

traced where the effect of AlP on the products of lipid peroxidation. (such as lipid peroxides & lipid hydroperoxides) was considered.

iii. Reactive oxygen species or oxygen-centered radicals damage the cell. a precise nature of oxygen radicals produced in the brain & spinal cord was unclear. The precise nature of oxygen radicals produced in the brain & spinal cord was unclear. The presence of diffusible antioxidants provides protection against free radicals. Glutathione is essential for the protection of cells and protective enzymes such as SOD, GR, GSHPx and GST, are responsible for defense against free radical induced damage. There was no study traceable to evaluate the effect of AlP on these enzymes in the rat brain.

iv. Effect of passage of time on the levels of monoamine oxidase (MAO) has been studied. However, investigations related to the effect of AlP on the MAO are lacking.

v . Studies have been conducted to see the effect of senscence on the nucleic acids (DNA and RNA). DNA is central to genome and RNA is responsible



for protein synthesis. However, the effect of AlP on regional distribution of nucleic acids has not been studied in rat brain and spinal cord.

vi. Major constituents of the cellular organelles and biomembranes are proteins. Protein damage and an increased role of intracellular proteolysis by phosphine have been subjected critical study. However, we could not find any such study where the effect of AlP have been studied in CNS of rat.

vii . To estimate the level of AChE. AChE is a neurotransmitter and responsible for transmission of message. However, information on the effect of AlP on AChE level in rats was not available.

viii. Vitamin E (α-Tocopherol) inhibitor of free radicals. The effect of vitamin E on various regions of CNS in rats following AlP intoxication were not studied to date.

2.32 The Scope of the Present Study :

AlP is extensively used as a fumigant by farmers. It is especially used for the protection of seed grains from nematodes. Hypoxia has been claimed to be a

leading cause of death in cases of AlP poisoning (Chaudhury, 1994). In hypoxia death of neurons occurs within 1 to 5 minutes. It has also been reported that in hypoxic condition, brain lipid level decreases. But the effects of AlP poisoning on lipid metabolism, lipid peroxidation lipid hydroperoxidation and SOD activity have not been evaluated. It would be worth investigating whether like the organophosphate compounds, the inorganic AlP also possess any cholinestrase inhibiting activity and Glutathione metabolism.

It has been reported that AlP is as toxic as KCN but no antidote is yet available against it. In proposed research to undertake indepth study of the machanism of neurotoxicity of AlP with the view of find out a suitable antidote has been undertaken. Earlier, Tayabba et al. (1985) from this laboratory have successfully demonstrated the efficacy of vitamin E against metasystox (an organophosphate pesticide) toxicity.

The study is likely to open up new vistas regarding the effect of AlP and antioxidant, vitamin E (α-Tocopherol) on neurochemical mechanisms is different regions of the rat CNS.

MATERIAL & METHODS

Material and Methods

3.1 Materials

Male albino rats of Charles Foster Stain, weighing 200 ± 20 g were obtained from the Central Animal House of J. N. Medical College, A.M.U., Aligarh. Animals were maintained in a well aerated animal room. Food (standard rat feed pellets from Hindustan Lever Limited, Bombay) and water were provided ***ad libitum***.

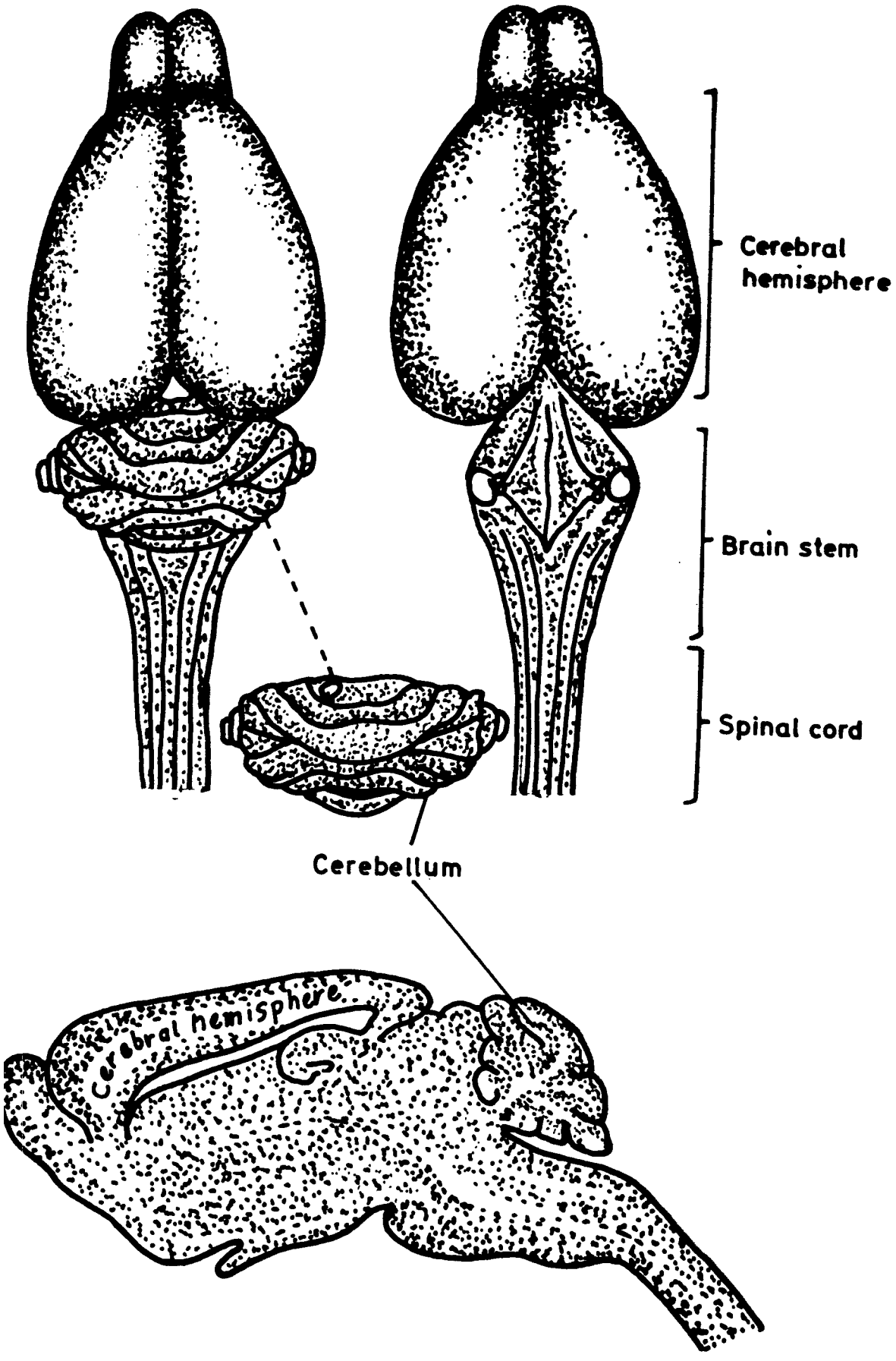
Pesticide

AlP (Qickphos(R), Celphos) was obtained from M/ s Bharat Seeds Store, Aligarh.

3.2 Methods

3.2.1 AlP Administration

AlP produce phosphine gas, which is highly toxic. The experimental animals were given the solution, orally, via a gavage. The volume administered was maintained at 2.0ml/kg body weight daily for 7 days.



Dissection of different parts of the rat CNS (McEwen and Pratt 1970.).

3.2.2 Treatment Protocol

The treatment protocol (dose given mg/kg body weight). number of doses (single dose), number of administations (repeated for 7 days). Animals were divided into four groups of six rats each. Group I, II & III were the experimental groups whereas rats of group IV served as control.

Group- I: A freshly prepared solution of aluminium phosphide (10 mg/kg, body weight) in physiological saline was given to rats of this group by gavage (into the pharynx) daily for 7 days.

Group- II: To the animals of group II aluminium phosphide (10 mg/kg b.wt.) in combination with α-Tocopherol (150 IU/kg b.w.) by gavage for 7 days.

Group- III: α-Tocopherol (150 IU/kg b.w.) Intraperitoneally daily for 7 days.

Group- IV: Animals served as control and an equal volume of physiological saline was given concurrently.

3.3 Behavioral Study:

Open Field Behaviour (OFB):

3.3.1 Apparatus

OFB apparatus used in present study was similar to that used by Holland and Gupta (1966). Briefly it consisted of a wooden, circular open arena (82 cm diameter) surrounded by a wall (31 cm high). The wooden floor was marked with three centric circles which were divided into segments by lines radiating from the centre. These 25 units of approximately equal size were used to score ambulation of the animals during the test. Two types of stimuli were presented to the animals: while noise (78dB, Ref. Intensity $2x10^{-4}$ dyn/ cm^2) was produced by an oscilator through four loud speakers; and light (165 FC) was shown by four lamps. A translucent glass screen enclosed the arena on all sides, the front side having a glass door through which the animals were placed in the arena.

3.3.2 Procedure

After one hour of treatment, each animal of both the treated and control groups was exposed daily for two

minutes in the apparatus, and the ambulation, preening and rearing responses were recorded by a three-channelled hand operated counter.

3.4 Ambulation

It is defined as the walking score derived from the number of radical segments crossed by the animals. The placement of all the four limbs in one segment was taken as one unit of ambulation.

3.5 Preening

Preening response was determined by the number of times the animal scratched its face with the forelimbs.

3.6 Rearing

A rearing score of one was awarded when the rat stood on its hind limbs with the support of the wall, and two for standing without support.

3.7 Neurobiochemical Studies:

The rats were killed after drug administration and the brain was removed immediately and placed on ice. Thereafter brain was dissected out into cerebrum,

cerebellum, brain stem and spinal cord, and each part homogenised in 10 ml chloroform : methanol (2:1, v/v) separately, but for the estimation of enzymes activity, the brains were homogenised in their respective buffer to give 10% (w/v) homogenate. The various neurochemical parameters were carried out according to the following methods.

Parameters	**Methods**
Total lipids (TL)	Woodman and Price (1972)
Cholesterol (CHL)	Zlatis et al., (1954)
Lipid peroxidation (LPO)	Okhawa et al., (1979)
Lipid hydroperoxidation (LHPO)	Haldebrandt and Roots (1975)
Total Sulfhydryl Groups (T-SH)	Sedlack and Lindsay (1968)
Free sulfhydryl group (GSH)	Sedlack and Lindsay (1968)
Glutathione oxidized (GSSG)	Folbergrova et al., (1979)
Superoxide dismutase (SOD)	Marklund and Marklund(1974)
Glutathione reductase (GR)	Hazelton and Lang (1985)
Glutathione peroxidase (GSHPx)	Lawrence and Burk (1976)
Glutathione-S-transferase (GST)	Habig et al., (1974)
Monoamine oxidase (MAO)	Tabor et al., (1953)
Nucleic Acids	
(i) DNA	Burton (1956)
(ii) RNA	Dische (1955)
Protein	Lowry et al. (1951)
Acetylcholinesterase (AChE)	Ellman et al., (1961)

3.8 Dissection of Brain into Discrete Regions: Cervical Dislocation

For biochemical studies, where perfusion of the rat brain was not required, the animals were killed by cervical dislocation, one of the most acceptable methods of euthanasia. The control as well as experimental rats were grasped at their neck near the base of skull, with the thumb and forefinger of one hand, and hindlimbs and tail with the other. A swift but controlled motion separated the cervical vertebrae from the base of skull. This resulted in instantaneous loss of consciousness and loss of all vital signs within a few minutes.

3.9. Extraction of Lipids from Discrete Brain Areas:

Different parts of the brain were homogenized in a glass homogenizer in chloroform- methanol (2:1 v/v) (40 mg/ml). Each homogenate was shaken periodically for an hour & filtered under vacuum through a sintered funnel (G-4). The residue of each test tube was again homogenized with 2.0 ml chloroform-methanol mixture and filtered. The test tubes were rinsed with fresh chloroform- methanol (2:1) and again filtered. The final volume of each extract was made to 10 ml with fresh chloroform methanol mixture. Thereafter, 2.5 ml of normal saline solution was added to the extract in each test tube (4:1 v/v). This was shaken vigorously on cyclomixer and placed at -20°C in a deep freeze overnight for complete separation of the two layers. The upper layer was used for the estimation of gangliosides and desired amount of the layer of each test tube was collected in stoppered tube with the help of syringe (long size) and stored at -20°C for 24 hours. The test tubes, in which the two layers were separated, were dried and the volume of the lower layer of each test tube was measured. The extract was used for the estimation of total lipids and cholesterol.

3.10. Estimation of Total Lipids:

Total lipids were estimated according to the method of Woodman and Price (1972) as follows:

Principle: Colour was developed with the help of colouring reagent (phospho-vanillin) in the presence of H_2SO_4 and O.D was read at 540 nm. H_2SO_4 acts upon the double bonds on lipids to produce carbonium ion which simultaneously reacts with phosphate ester of vanillin to form a colored complex.

Procedure: 0.1 ml of brain extract in duplicate was taken in 18 x 150 mm test tube. 2.5 ml of conc. H_2SO_4 was added to each test tube and heated on boiling water bath for 20 min. After cooling, 5.0 ml of colouring reagent (6.0g $K_2H_2PO_4$ and 0.39 g vanillin in 100 ml DDW) was added and absorption was read at 530 nm exactly after 10 min. against a reagent blank.

A calibration curve with different concentration (100-600 µg) of standard brain lipids (extracted from rat brain) was prepared by adopting the same procedure as described above.The values of the standard curve were

plotted by least squares method. The concentration of total lipids in brain samples were calculated by the following formula:

Calculation: Total lipids (mg/g fresh wt.)= $\frac{C \times V}{Vt. \times Wt.}$

where

C = concentration of lipids in µg in 0.1 ml extract

V = total volume of the lower layer

Vt = volume taken for the estimation

Wt = fresh weight of the tissue in mg.

3.11 Estimation of Cholesterol:

Cholesterol was estimated according to the method of Zlatis et al. (1954).

Principle: Cholesterol when dissolved with acetic acid and in the presence of $FeCl_3$-H_2SO_4 reagent gets dehydrogenated to 3,5-cholestadiene or 2,4-cholestadiene which simultaneously polymerizes and reacts with $FeCl_3$ to form a violet colour complex which is measured spectrophotometrically at 570 nm.

Procedure: 0.05 ml of different parts of brain extract in duplicate were taken in test tubes, dried and dissolved in 3.0ml of glacial acetic acid. Then 2.0 ml of working $FeCl_3$ was added and the contents were mixed thoroughly. The tubes were kept in dark for 30 minutes and the O.D was then measured at 570 nm. Reagent blank and standard cholesterol solution (1.0 mg/ml) were also run simultaneously.

Calculation

$$\text{Cholesterol (mg/g tissue weight)} = \frac{C \times V}{Vt \times Wt}$$

3.12. Estimation of Lipid Peroxidation (LPO) :

Rate of LPO was estimated by the method of Okhawa et al. (1979).

Principle: Lipid and protein of brain tissue is detached by action of acetic acid. The protein in the reaction mixture is dissolved by the addition of sodium dodecyl sulphate. TBA reacts with lipid peroxide, hydroperoxide and oxygen labile double bond to form the colour adducts.

Procedure: 0.2 ml of each sub cellular fraction (in 10% w/v of 1.5M KCl) was mixed with 1.0 ml of 20% acetic acid. Subsequently 0.2 ml of 8.0% aqueous SDS was mixed. After this, 1.5 ml of 0.8% TBA (pH-7.0) and 1.1 ml of DDW were added (final volume was 4.0 ml). The above reaction mixture was incubated in a boiling water bath for an hour. After cooling to room temperature, 3.0 ml of n-butanol was mixed in each test tube. The reaction mixture was then centrifuged at 10,000 x g for 15 minutes. A clear butanol supernatant was used for measuring the O.D. at 532 nm against a reagent blank.

Calculation: Standard absorbance of MDA (2.5 n mol) was used to calculate the amount of lipid peroxide in the samples and results were expressed as n mole of MDA/ g tissue weight.

3.13 Estimation of Lipid Hydroperoxidation (LHPO):

LHPO was estimated by the method of Haldebrandt and Roots (1975).

Principle: This method is based on the formation of $Fe(SCN)_3$ from ferrous ammonium sulphate and

potassium thiocyanate on peroxidation of Fe^{2+} to Fe^{3+} by H_2O_2 which results into the development of an intense pale colour.

Procedure: 2.0 ml of brain homogenate (in 0.15M KCl, 10% w/v) was treated with 1.0 ml of 20% TCA. It was incubated at 0°C for 30 minutes and there after centrifuged at 14,000 x g for 30 minutes in cold. 1.0 ml of the clear supernatant was carefully mixed with 0.2 ml of 10 mm $Fe(NH_4)_2(SO_4)_2$ and 0.1 ml of 2.5M KSCN. The tubes were shaken thoroughly, kept for 10 minutes and then O.D was read at 480 nm against a reagent blank.

Calculation: Standard absorbance of cumin hydroperoxide (4.0n mol) was used to calculate the amount of lipid hydroperoxide in the samples and results were expressed as n mol of cumin hydroperoxide per g.wt. tissue.

3.14 Estimation of Total Sulfhydryl Groups (T-SH):

T-SH was estimated following the method of Sedlak and Lindsay (1968).

Principle: 5-5'-dithiobis-2-nitrobenzoic acid (DTNB) is reduced by -SH groups of glutathione (GSH) in alkaline medium to produce one mole of 2-nirto-5-mercaptobenzoic acid per mole of -SH group.Since the anion (2-nitro-5-mercaptobenzoic acid) has an intense yellow colour, it can be used to measure -SH group at 412 nm.

Procedure: Various parts of the brain were homogenized in chilled 0.15M KCl (10% w/v). In 0.1 ml brain homogenate, 1.5 ml of 0.2 M Tris–Succinic acid buffer (pH 8.2) and 0.1 ml of 0.01 M DTNB were added (total volume was 1.7 ml). The mixture was shaken and volume made up to 10 ml with 8.3 ml of absolute methanol. The reaction mixture was centrifuged at 6,000x g for 5 minutes in cold. The O.D of the clear supernatant was read at 412 nm. A calibration curve with different concentrations of GSH (200-1600 μ moles) was obtained according to the same procedure as described above. The values were plotted by least squares method.

Calculation Total -SH group in the samples were calculated using the standard curve and the results were expressed as μ moles/g tissue.

3.15 Estimation of Free Sulfhydryl Group (GSH):

GSH was estimated by the method of Ellman (1959) as modified by Sedlak and Lindsay (1968).

Principle: Same as for total sulfhydryl group estimation.

Procedure: Different brain parts were homogenized (10% w/v) in chilled 0.15M KCl. Take 1.0 ml brain homogenate and add 1.0 ml of 10% TCA and then centrifuged at 6,000 x g for 5 minutes. 0.5 ml clear supernatant was mixed with 0.5 ml DDW. Thereafter, 2 ml of 0.4 M Tris-EDTA buffer (pH 8.9) and 0.1 ml of 0.01 M DTNB were added to it with stirring. The O.D was read at 412 nm within 5 minutes of the addition of DTNB.

Calculation: Free -SH (GSH reduced) in the samples were calculated using the standard curve of GSH (200-1600 μ moles and the results were expressed as μ moles/ g tissue.

3.16 Estimation of Oxidized Glutathione (GSSG):

GSSG was estimated by the method of Folbergrova et al. (1979).

Principle: The estimation of GSSG by enzymatic method is based on the reduction of GSSG in the presence of NADPH and glutathione reductase and the determination of the decrease in NADPH absorbance at 340 nm.

$$GSSH + NADPH \xrightarrow[H^+]{GR} NADP^+ + GSH$$

Procedure: The brain parts were homogenized in ice-cold 1.0 mM EDTA solution (10% w/v). An aliquot of the homogenate was immediately transferred to tubes containing 0.1 ml of 0.05 M NEM and allowed to stand for 10 minutes at 0°C after mixing. The proteins were precipitated by the addition of 1.0 ml of ice-cold 12.0% TCA giving a final concentration of approximately 5.0% TCA. After thorough mixing the suspension was centrifuged at 0°C for 10 min at 3,000 rpm. The supernatant was extracted four times with solvent ether to remove TCA and NEM. Aliquots of supernatant were

then used for GSSG analysis. The reaction mixture consisted of 0.5 ml of 100 mM imidazole (glyoxaline) HCl buffer (pH 7.5), 0.2 ml of 5.0 mM EDTA, 0.1 ml of 0.02% BSA, 0.1 ml of 0.002 mM NADPH and 0.1 ml cytosolic supernatant (total volume was 1.0 ml). The reaction was initiated by the addition of glutathione reductase (0.1 Unit per ml). The initial O.D was measured at 340 nm before addition of enzyme and the final readings were made when the reaction was complete (5-10 minutes). Standard of GSSG (15 milli mole solution, range of 1.5 $x10^{-10}$ -10^{-9}) was also run simultaneously.

Calculation: GSSG content in the samples was calculated using the standard curve and the results were expressed in μ mol GSSG/g of wet tissue.

3.17 Estimation of Superoxide Dismutase (SOD):

SOD activity was measured by the method of Marklund and Marklund (1974).

Principle: SOD principle depends upon autoxidation of pyrogallol.

$$\text{Pyrogallol} + O_2 \xrightarrow{\text{autoxidation}} \text{oxidation product} + O_2^- \quad \text{(i)}$$

$$2O_2^- + 2H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2 \quad \text{(ii)}$$

Procedure: Different CNS parts (cleaned with normal saline) were homogenized in chilled 0.15M KCl (10% w/v). Homogenate was centrifuged in cool at 10,000 rpm for 15 min. A 0.05 ml of clear supernatant was added to 2.85 ml of 0.05 M Tris- succinate buffer (pH 8.2), mixed well and incubated at 25°C for 20 min. The reaction was started by adding 0.1 ml of 8 mM pyrogallol solution. The contents were shaken well and change in O.D per min. was immediately recorded for 3 min. at 420 nm. A reference set, consisting of 0.05 ml of DDW instead of the sample solution (clear supernatant) was also run similarly.

Calculation

$$\text{SOD} = \frac{(\text{A/min. ref} - \text{A/min. sample}) \times 30}{(\text{A/min. ref}/2 \times 0.05 \times 1)} \text{ units/10mg tissue}$$

Where,

A/min. ref. = change of O.D per min. in reference set

A/min. sample = change of O.D per min. in sample set.

Activity Unit: One unit of the enzyme is defined as the amount of enzyme which causes a 50% inhibition of pyrogallol autoxidation under assay conditions.

3.18 Estimation of Glutathione Reductase (GR):

Glutathione reductase was assayed by the method of Hazelton and Land (1985).

Principle: Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) by NADPH to reduce glutathione (GSH) according to the following equation:

$$GSSG + NADPH + H^{+} \xrightarrow{GR} NADP^{+} + GSH$$

The activity of the enzyme was measured by following the decrease in optical density/minute at 340 nm during oxidation of NADPH.

Procedure: The reaction mixture consisted of 0.1 ml of 0.1 mM NADPH, 0.2 ml of 3.0 mM GSSG, 0.1 ml of 1.0 ml EDTA, 2.5 ml of 0.1M Tris-HCl buffer (pH 8.0) and 0.1 ml tissue supernatant (10% w/v in Tris-HCl buffer; enzyme source) in a total volume of 3.0 ml. The

reaction was initiated by the addition of tissue supernatant. Oxidation of NADPH was followed at 340 nm. Reference reaction was also run simultaneously. Protein content in enzyme source was also determined (Lowry et al. 1951). Increase in optical density/minute was deduced.

Calculation: Enzyme activity was calculated using the molar extinction coefficient of NADPH (6.22×10^3 M^{-1} cm^{-1}) and results were expressed as n mole of NADPH oxidized per minute per mg protein.

3.19 Estimation of Glutathione Peroxidase (GSHPx):

GSHPx was assayed according to the method of Paglia and Valentine (1967) modified by Lawrence and Burk (1976).

Principle: It measures the rate of GSH oxidation by H_2O_2 as catalyzed by the GSHPx present in the tissue supernatant. The substrate is maintained at a constant concentration by the addition of oxogenous GSSG-R and NADPH, which immediately converts any GSSG produced to the reduced form (GSH).

$$2GSH+H_2O_2 \xrightarrow{GSHPx} H_2O+GSSG \xrightarrow[2NADPH]{GSSG\text{-}R} 2GSH+2NADP^+$$

The rate of GSSG formation is then measured by following the desrease in absorption of the reaction mixture at 340 nm as NADPH is converted to $NADP^+$.

Procedure: The reaction mixture consisted 0.5 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.3 ml of EDTA (1.0 mM), 0.4 ml of NaN_3 (1.0 mM), 0.1 ml of NADPH (0.2 mM), GSSG-R (purified enzyme) (1.0 E.U.), 0.1 ml of GSH (1.0 mM), 0.2 ml of H_2O_2 (0.25 mM) and 0.9 ml of tissue supernatant (10% w/v in phosphate buffer; total volume is 2.5 ml). All ingredients except enzyme source (tissue supernatant) and H_2O_2 were combined at the begining of experiment. Tissue supernatant was added to the above mixture and allowed to incubate for 5 minutes at room temperature. The reaction was initiated by addition of H_2O_2. Optical dencity at 340 nm was recorded for 5 minutes after 30 seconds interval. Blank reaction with enzyme source replaced by distilled water was also carried out to find out the non enzymatic change, if any. Protein content in enzyme source was also determined.

Calculation: Enzyme activity was calculated on the basis of molar extinction coefficient for NADPH ($6.22x10^3M^{-1}$ Cm^{-1}). Results were expressed as n moles NADPH oxidized/min/mg protein.

3.20 Estimation of Glutathione-S-Transferase (GST):

Glutathione-S-Transferase (GST) activity was assayed by the method of Habig et al. (1974).

Principle: The enzyme activity is measured by following the increase of absorbance at 340 nm of CDNB-GSH conjugate generated as a result of GST catalysis between glutathione and 1-chloro-2, 4-dinitrobenzene (CDNB).

$$CDNB + GSH \xrightarrow{GST} CDNB - GSH \text{ (conjugate product)}$$

In 0.1 ml of cytosol fraction (supernatant) (10% w/v in 0.15M KCl), 2.7 ml of 1.0 mM glutathione solution (prepared in 0.2 M phosphate buffer) and 0.2 ml CDNB (1.0 mM) substrate prepared in acetone were mixed. The change in absorbance at 340 nm was recorded at room temperature after 15 sec. for 3 min.

against a blank containing 0.1 ml DDW in place of supernatant. Protein content in enzyme source was also determined.

Calculation

$$\text{GST Activity} = \frac{\text{O.D x 625}}{\text{Conc.of protein (mg)}} \longrightarrow \text{U/mg/min/protein}$$

The values were calculated on the basis of molar extinction coefficient of CDNB ($9.6x10^3\ M^{-1}\ cm^{-1}$) and specific activity of enzyme was expressed in n mole of GSH- CDNB conjugate formed per minute per mg protein.

3.21 Estimation of Momoamine Oxidase (MAO):

MAO activiy was determined by the method of Tabor et al. (1953).

Principle: The benzylamine undergoes oxidative deamination in the presence of MAO and benzaldehyde is formed.

$$R\text{-}CH_2CH_2NH_2 + O_2 + H_2O \longrightarrow R\text{-}CH2CHO + H_2O_2 + NH_3$$

Procedure: The reaction mixture in a final volume of 2.0 ml consisted of 0.4 ml of 0.5 M phosphate buffer (pH 7.2), 0.1 ml of M benzylamine hydrochloride and 0.2 ml of brain homogenate (10% w/v in phosphate buffer). The reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by the addition of 1.0 ml of 10% TCA (proteins were precipitated). The above reaction mixture was centrifuged at 2,500 rpm for 10 minutes in cold. The optical density of benzaldehyde formed was read in the supernatant at 250 nm against the blank containing 0.2 ml of 0.44 M sucrose instead of brain supernatant. Protein concentration were also determined by the method of Lowry et al.(1951).

Calculation

$$\text{Activity of MAO} = \frac{15.385 \times \text{OD/min}}{\text{protein cons. (mg)}}$$

The activity of MAO was expressed as n moles benzaldehyde formed/min/mg protein.

3.22 Nucleic Acids:

Principle: The procedure for the determination of nucleic acids described below is based on the finding that nucleic acids can be separated from other tissue compounds by their preferential solubility in hot TCA or PCA. The isolated nucleic acids are then quantitated by means of spectrophotomeric reactions involving the pentose compounds of the nucleic acids.

Isolation of Nucleic Acids:

Nucleic acids were isolated following the method of Searchy and Macinnis (1970). Weighed tissue of the different brain regions were homogenized (5% w/v in 0.5 N $HClO_4$). The homogenates were boiled on water bath for 10 minutes, cooled and centrifuged at 3,000 x g for 10 minutes. Supernatants were taken in graduated test tubes and the volume was maintained upto 5.0 ml (with 0.5 N perchloric acid). This extract was used in the estimation of DNA and RNA according to the following procedures:

3.22.1 Estimation of DNA:

DNA was estimated following the method of Burton (1956).

Principle: Deoxyribose is converted into highly reactive hydroxy vulnaldehyde, which reacts with diphenyl amine (DPA) to give a blue coloured complex.

Deoxyribose sugar+DNA ——————>hydroxy vulnaldehyde

Procedure: 2.0 ml of the perchloric acid supernatant of nucleic acid extract was taken in a test tube. To this 4.0 ml diphenylamine (1.5% in CH_3COOH-H_2SO_4) reagent was added and the tubes were heated on boiling water bath for 15 minutes. After cooling, the colour intensity was measured at 600 nm against a blank sample (2.0 ml DDW in place of supernatant). A standard curve was prepared by using standard solutions mg/ml in 0.5 N $HClO_4$ of DNA (100 to 600 µg) according to the same procedure. The values were plotted by the least square method.

Calculation

$$\text{DNA quantity} = \frac{C \times V}{Vt \times Wt}$$

Where,

C = Conc in mg (in 2.0 ml extract)

Vt = Volume taken for the estimation

Wt = Fresh weight of the brain in mg

V = Total volume of the extract (4.0 ml)

DNA in the reaction product was calculated using the standard curve of DNA that was run simulatneously with the test sample. Results were expressed as mg DNA/g fresh tissue weight.

3.22.2 Estimation of RNA:

RNA was estimated by the method of Dische (1955)

Principle: Pentose sugars are converted to furfural derivatives by heating with conc. HCl. In the presence of $FeCl_3$ solution the furfural derivatives react with orcinol and produce a green colored complex.

Procedure: 2.0 ml of the perchloric acid supernatant of nucleic acid extract was taken in a test tube, 4.0 ml of the orcinol reagent (33.0 mg FeCl3 + 3.5 ml of 6% orcinol in 100ml HCl) was added to it. Test tubes were heated on boiling water bath for 15 minutes, cooled and the absorbance was read at 660 nm against a reagent blank (2.0 ml of DDW in place of sample). A calibration plot with different concentrations of RNA (100-600 µg) was drawn according to the same procedure as described above.

Calculation: Same as for DNA

RNA content in the samples was calculated using the standard curve. The results were expressed as mg RNA per g fresh tissue weight.

3.23 Estimation of Protein:

Protein estimation was done by the method of Lowry et al. (1951).

Principle: This method is based on colour reactions of amino acids tryptophan and tryosine with Folin phenol reagent. By the reaction of these amino acids with phosphomolybdic acid and phosphotungstic acid (Present

in Folin's reagent), a blue colour is formed. O.D was read at 625 nm. The colour is the result of reduction of phosphomolybdic acid and biuret reaction of proteins with Cu^{++} ions in alkaline medium.

Reagents:

(i) Copper regent

A: 4.0% sodium carbonate in DDW

B: 2.0% copper sulphate in DDW

C: 4.0% (w/v) sodium-potassium tartrate in DDW

Mixed A, B and C reagents in a ratio of 100:1:1 respectively at the time of use.

(ii) Folin-Ciocalteau phenol reagent

2 N solution obtained commercially was diluted 1:1 with double distilled water before use.

(iii) Standard bovine serum albumin (BSA) (1 mg/ml). Stock standard was diluted ten times to get the working standard of 100 µg/ml.

Procedure:To 0.1 ml of tissue aliquot (residue left in the test tube after shaking was added to the

supernatant for nucleic acid estimation, was dissolved in 5.0 ml DDW).To this 5.0 ml of copper reagent was added and shaken well and incubated at 37°C. After 10 minutes 1.0 ml of Folin-Ciocalteau reagent was added. Optical density of the blue colour developed was read at 625 nm *exactly after 30 minutes. Standard protein solution* (BSA, 20-100 µg) and blank were also run simultaneously.

3.24 Estimation of Acetylcholinesterase (AChE):

AChE was assayed according to the method of Ellman, et al. (1961).

Principle: AChE estimation is based on the measurement of the rate of production of thiocholine as AChE is hydrolysed. This is accompanied by the yellow anion of 5-thio-2-nitrobenzoic acid.

Procedure: Different parts of brain tissue were homogenized in 0.1M phosphate buffer (10 mg/ml; pH 8.0) and centrifuged at 1,500 rpm in cold for 5 min., 0.4 ml of DTNB reagent (39.6 mg DNTB and 15.0 mg

of $NaHCO_3$ in 10 ml of 0.1M phosphate buffer) was added and mixed well. O.D was read at 412 nm. 0.02 ml of the substrate was added and the changes in the O.D were recorded from 5th to 10th min at the interval of one minute. To determine non-specific esterase, 0.1 ml of eserine sulphate (0.1 mM; dissolved in 0.1 M phosphate buffer) was added to 0.4 ml of tissue supernatant, 2.5 ml of phosphate buffer and 0.1 ml of DTNB reagent. The changes in O.D were recorded as described above, after adding 0.02 ml of substrate (0.075 M acetylthiocholine iodide). The enzyme activity was expressed as u moles of substrate hydrolysed per g tissue per minute.

Calculation

$$\text{Rate of enzyme activity (R)} = \frac{A}{1.36 \times 10^4} \times \frac{1}{(400/3120/Co}$$

$$R = 5.74 \times 10^{-4} \frac{A}{Co}$$

A = Change in absorbance per min.

Co = Original concentration of tissue (mg/ml)

RESULTS

RESULTS

4.1 Physical Sign and Symptoms of AIP Toxicity:

AIP intoxication was found to produce physical signs such as dribbling of saliva from mouth, tachyphnoea, restlessness followed by lerharginess and drowsiness. Diarrhoea, dizziness and tremors were also observed in experimental animals. Weight of the treated rats also decreased.

4.2 Calibration of Standard Curves:

The standard curves of different known concentrations of the total lipids, cholesterol, DNA, RNA, protein, MAO, GSSG and Glutathione reduced (GSH) were calibrated against their O.D., respectively. The concentration of various lipid fractions was calculated from individual standard curves.

4.3 Open Field Behaviour (OFB) Study:

OFB study was also observed with AIP *toxicosis on* OFB parameters, such as

(i) Ambulation (ii) Preening (iii) Rearing

4.3.1 Ambulation:

Singnificant depletion ($p<0.001$) was observed from the third day of toxication . On the seventh and last day, maximum decrease was noticed (-94.5%) Table-1.

4.3.2 Preening:

The preening also showed significant decrement ($p<0.001$) on third day and also maximum on last day of toxicosis (-71.4%) Table-2.

4.3.3 Rearing:

The rearing score, was reported to show significant depletion ($p<0.001$) from the fifth day. On seventh day maximum decrease was observed (-83.8%) Table-3

Protective effect of vitamin E (150 IU/kg b.wt.,ip) on ambulation, preening and rearing were observed (Fig.1.1,2.1 & 3.1)

Table - 1

Perturbation in the ambulation score of the rats treated with Aluminium Phosphide (10mg/kg b.wt., gavage) daily for 7 days: Protection by α-Tocopherol

Three observations daily for 6 rats								
Control		Days	Experiment-1 (AlP)			Expreiment-2 (AlP+Vit. E)		
Mean	±SE		Mean	±SE	%change	Mean	±SE	%change
		0	16.950	0.300	NS	17.080	0.12	NS
		1	16.986	0.240	NS	17.125	0.15	NS
17.380	0.640	3	13.450[a]	0.380	-22.6	17.206	0.45	NS
		5	7.398[b]	0.160	-57.4	17.309	0.50	NS
		7	0.950	0.300	-94.5	17.092	0.21	NS

±SE = Standard Error; NS = not significant; (a) p< (0.01);(b) p< (0.001)

Ambulation Score Protective by Vitamin-E (150 IU/kg.b.wt.,ip.)

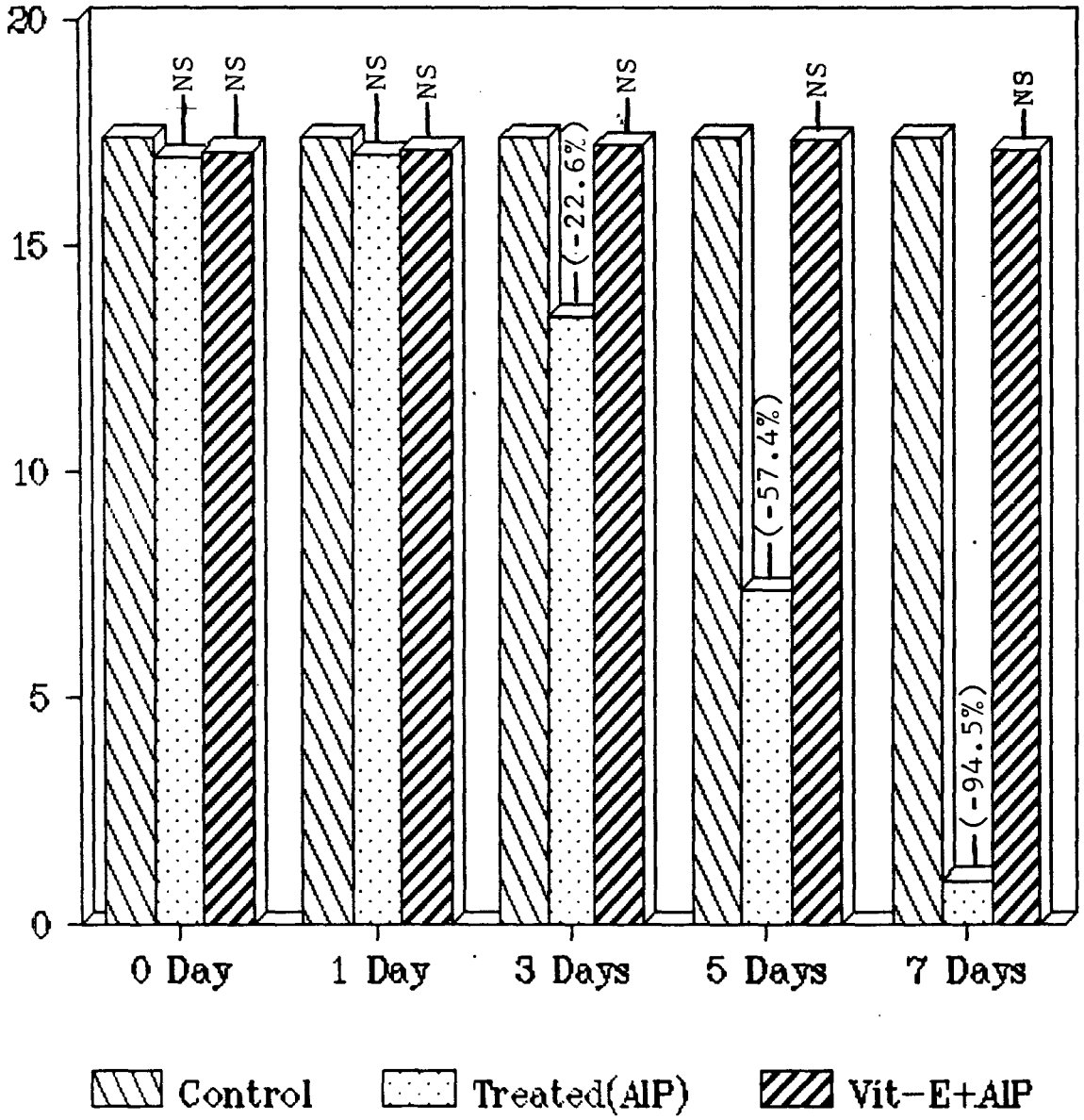


Fig. No. 1.1

Table - 2

Perturbation in the Preening score of the rats treated with Aluminium Phosphide (10mg/kg b.wt., gavage) daily for 7 days : Protection by α-tocopherol

Three observations daily for 6 rats								
Control		Days	Experiment-1 (AlP)			Expreiment-2 (AlP+Vit. E)		
Mean	±SE		Mean	±SE	%change	Mean	±SE	%change
14.45	0.910	0	14.15	0.39	NS	14.06	0.64	NS
		1	14.20	0.65	NS	14.38	0.59	NS
		3	10.26[a]	0.45	-28.9	15.06	0.74	NS
		5	9.36[b]	0.74	-35.2	13.95	0.48	NS
		7	4.13[b]	0.65	-71.4	11.90	0.36	NS

±SE = Standard Error;NS = not significant; (a) p< (0.01);(b) p< (0.001)

Preening Score Protective by Vitamin-E (150 IU/kg.b.wt.,ip.)

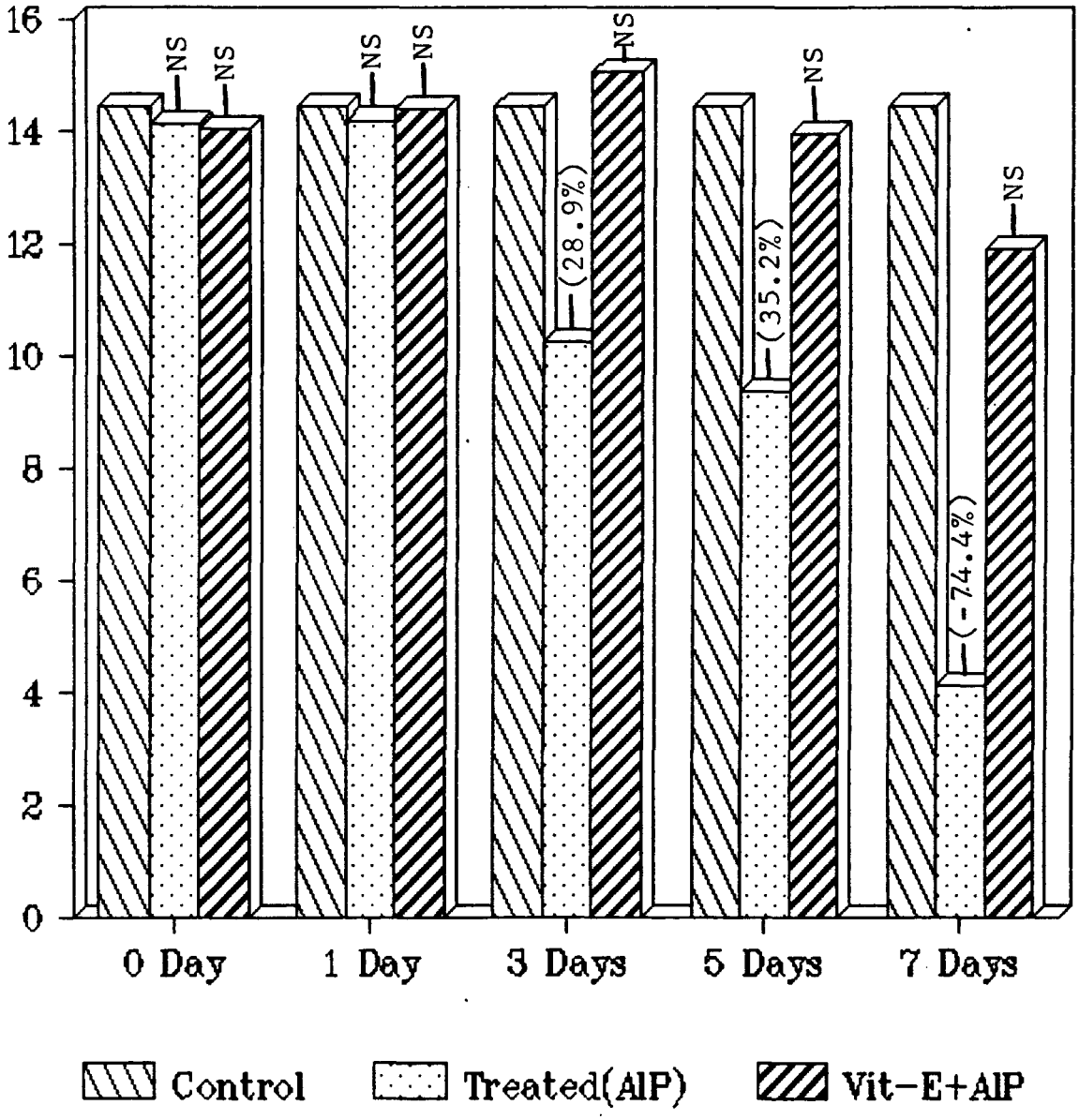


Fig. No. 2.1

Table -3

Perturbation in the rearing score of the rats treated with Aluminium Phosphide (10mg/kg b.wt., gavage) daily for 7 days: Protection by α-Tocopherol

Three observations daily for 6 rats								
Control		Days	Experiment-1 (AlP)			Expreiment-2 (AlP+Vit. E)		
Mean	±SE		Mean	±SE	%change	Mean	±SE	%change
		0	12.54	1.52	NS	12.39	0.69	NS
		1	12.34	0.63	NS	12.085	1.11	NS
12.45	1.25	3	10.09[a]	0.51	-18.9	12.35	1.20	NS
		5	5.26[b]	0.42	-57.7	12.40	0.96	NS
		7	2.01[b]	0.290	-83.8	11.965	0.90	NS

±SE = Standard Error;NS = not significant ; (a) $p < (0.01)$;(b) $p < (0.001)$

Rearing Score Protective by Vitamin-E (150 IU/kg.b.wt.,ip.)

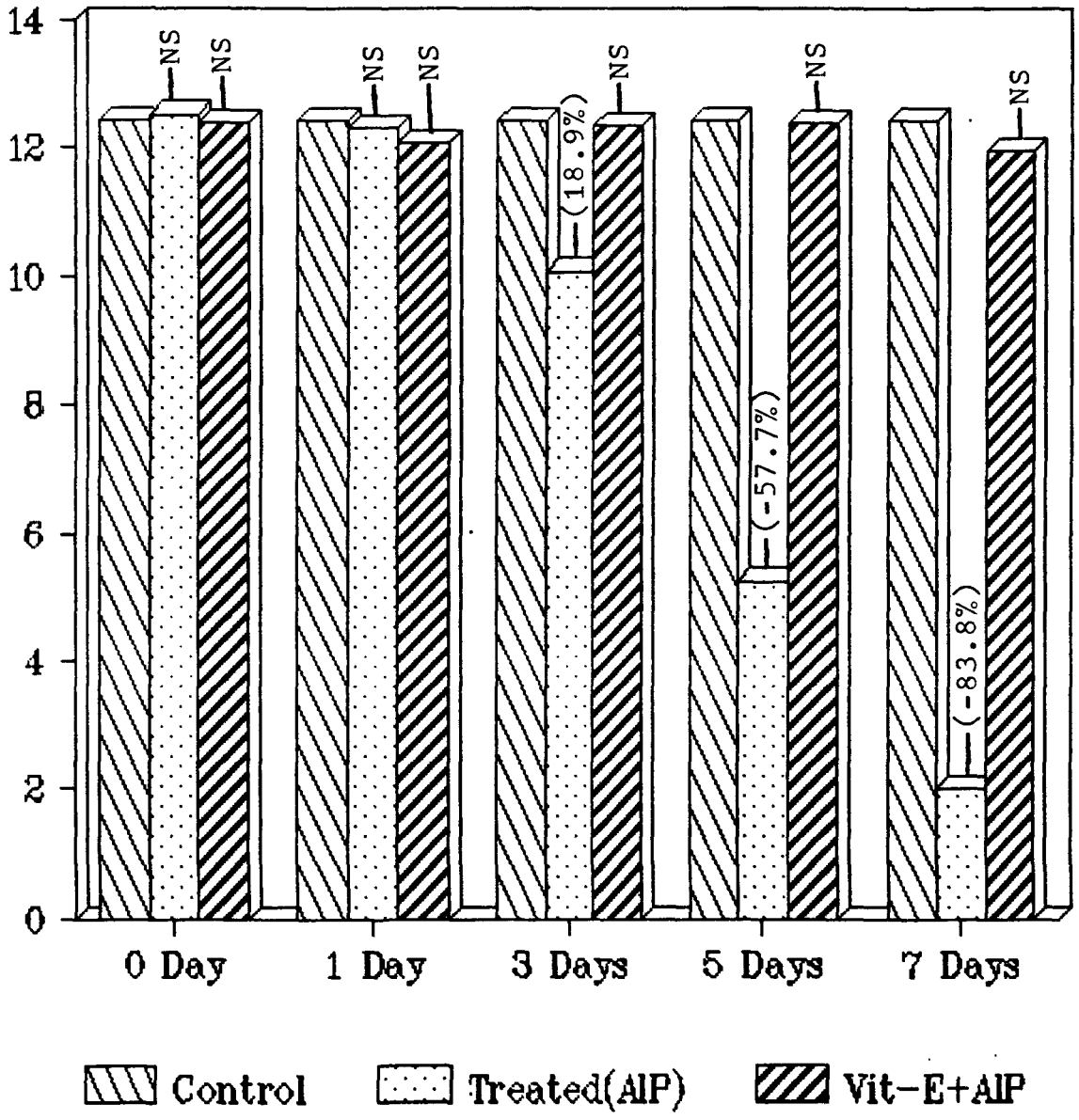


Fig. No. 3.1

4.4 Neurobiochemical Observations:

The following neurochemical parameters, were chosen to determine how closely related are the regional changes which occur in different regions of central nervous system, following AIP toxicosis The protective effect of a-tocopherol (vitamin E) was also observed.

4.4.1 Brain Lipids:

The results are presented in tables 4 and 5. The AIP associated alterations of total lipids and cholesterol in the different regions of the rat brain and spinal cord after 7 days intoxication are given below.

4.4.2 Total Lipids:

The total lipids level in different regions of brain and spinal cord are described (table 4). The concentration of total lipids showed the rank order was CBL>CBM>BS>SPC.

After toxicosis of AIP solution the content of total lipids were decreased significantly in cerebellum, cerebrum, brain stem and spinal cord by -40.22%, -39.62%, -31.86% and -24.59%, respectively.

Table 4

Alteration of Total Lipids in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, gavage) daily for 7 days.

Total Lipids content (mg/g tissues) (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	141.30	4.32	101.20	6.35	-39.62	<0.001
CBL	139.20	2.10	99.27	3.62	-40.22	<0.001
BS	190.23	8.93	144.26	4.73	-31.86	<0.01
SPC	240.85	5.45	193.30	5.40	-24.59	<0.05

±SE = Standard Error; n = no. of rats.

4.4.3 Cholesterol:

AlP intoxicosis led to remarkable depletion in the levels of cholesterol (table 5) in the cerebrum, cerebellum and spinal cord by -16.47%, -13.16% and -11.65%, respectively. Maximum progressive inhibition of cholesterol was in cerebrum (-16.47%), while non significant change in brain stem (-10.345).

4.4.4 Lipid Peroxidation:

Effect of AlP: The levels of the rate of lipid peroxidation was reported to increase significantly in various regions of the brain and spinal cord,after the treatment with AlP (Table-6). Values were found to increase in the following order: Cerebellum> Cerebrum> Spinal cord> Brain stem

by +22.11%, +21.16%, +18.89%, respectively.

Effect of Vitamin E: Interestingly, when vitamin E (150 IU/Kg b.wt,ip) was injected simulataneously with AlP, a significant depletion in the lipid peroxidation level was observed in various regions of brain and spinal cord of experimented rats (Fig.6.1). The rate of lipid

Table 5

Alteration of Cholesterol in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, gavage) daily for 7 days.

The levels of Cholesterol (mg/g tissues) (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	20.40	0.70	17.04	0.75	-16.47	<0.01
CBL	21.35	0.68	18.54	0.62	-13.16	<0.05
BS	28.02	0.79	25.18	0.80	-10.34	NS
SPC	36.20	1.25	31.98	0.86	-11.65	NS

±SE = Standard Error; NS = Not significant; n = no. of rats.

Table 6

Alteration of Lipid peroxidation in different regions of the rat CNS after the exposure to Aluminium Phosphide (10 mg/Kg.b.wt, gavage) daily for 7 days.

Lipid peroxidation n mol of MDA/g tissues weight (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	326.00	14.3	395.00	8.43	21.16	<0.001
CBL	377.42	16.9	460.90	12.80	22.11	<0.001
BS	314.70	1.08	371.50	13.40	18.04	<0.01
SPC	322.50	15.4	383.45	14.60	18.89	<0.01

±SE = Standard Error; n = no. of rats.

Protective Role of Vitamin-E on the rate of LPO (150 IU/kg.b.wt., Orally)

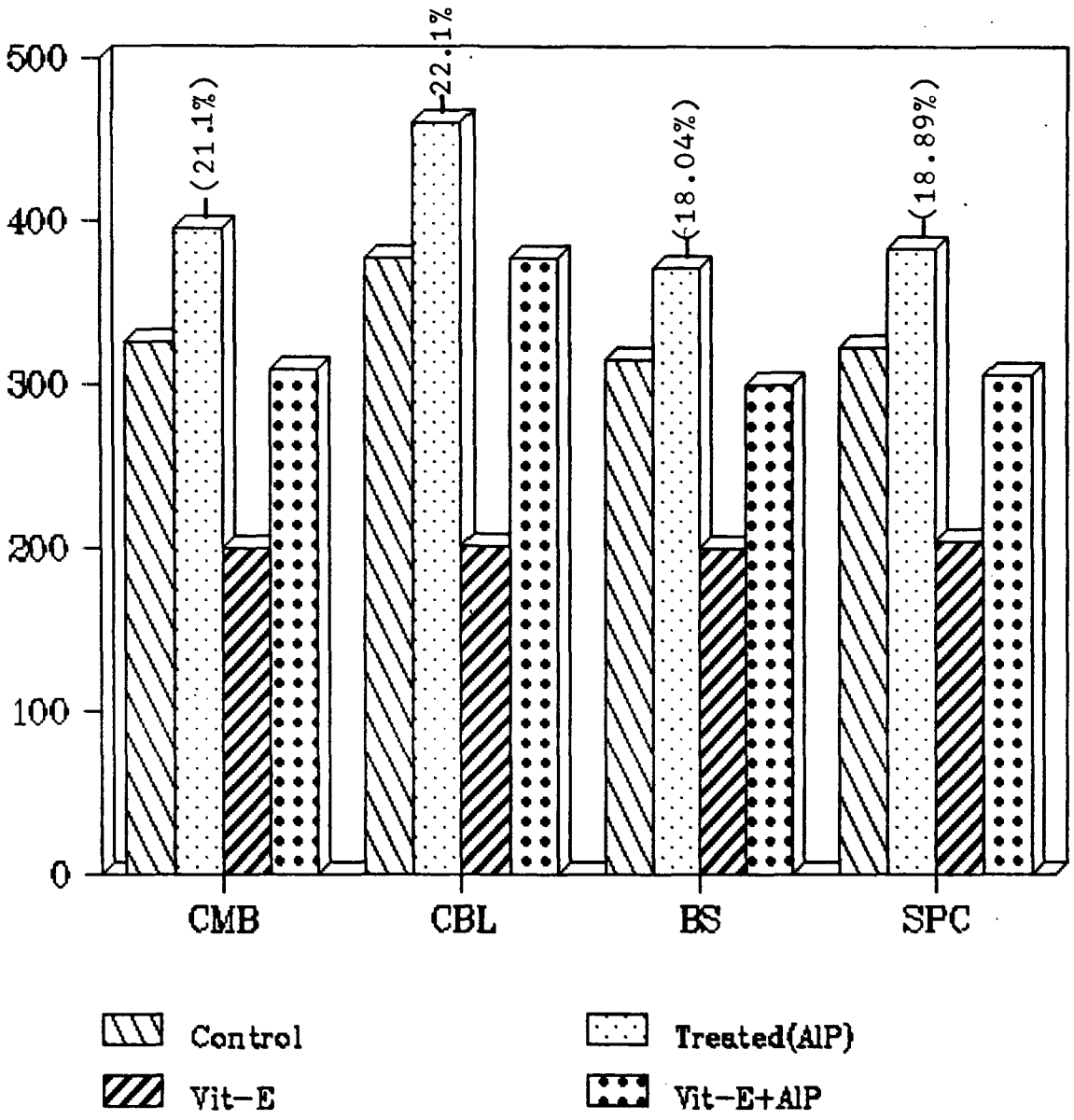


Fig. No. 6.1

peroxidation was observed to be near the control values in different regions of CNS.

4.4.5 Lipid Hydroperoxidation:

Effect of AlP: Table 7 shows the levels of LHPO found in different regions of brain and spinal cord. The levels of LHPO were higher in CBL by +26.86%. In other regions, the rank order was: Spinal cord> Brain stem> Cerebrum.

Effect of Vitamin E: When vitamin E (150 IU/Kg b. wt ip, for 7 days) was given, a significant change in the values of LHPO was observed in various regions of brain and spinal cord of rats when compared with their respective controal values (Fig.7.1).

4.5 Antioxidant System:

4.5.1 Total -Sulfhydryl Groups (T-SH):

The concentration of T -SH groups was reported to be significant in CBL and BS but less siginigicant in CBM and SPC (table 8).

The maximum depletion was observed in cerebellum (-26.24%) followed by brain stem (-24.81%).

Table 7

Alteration of Lipid Hydroperoxidation in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt., gavage) daily for 7 days.

The levels of LHPO (n mol of cumine Hydroperoxide/g tissue (n=6)						
Regions	Control		Treated			
	Mean	±SE	Mean	±SE	% Change	pValue
CBM	18.968	0.89	22.456	1.45	13.38	<0.05
CBL	27.954	1.25	35.463	1.96	26.86	<0.001
BS	17.116	0.85	20.989	1.60	22.62	<0.01
SPC	21.589	1.21	26.486	1.58	22.70	<0.01

±SE = Standard Error; n = no. of rats.

Protective Role of Vitamin-E on the rate of LHPO (150 IU/kg.b.wt., Orally)

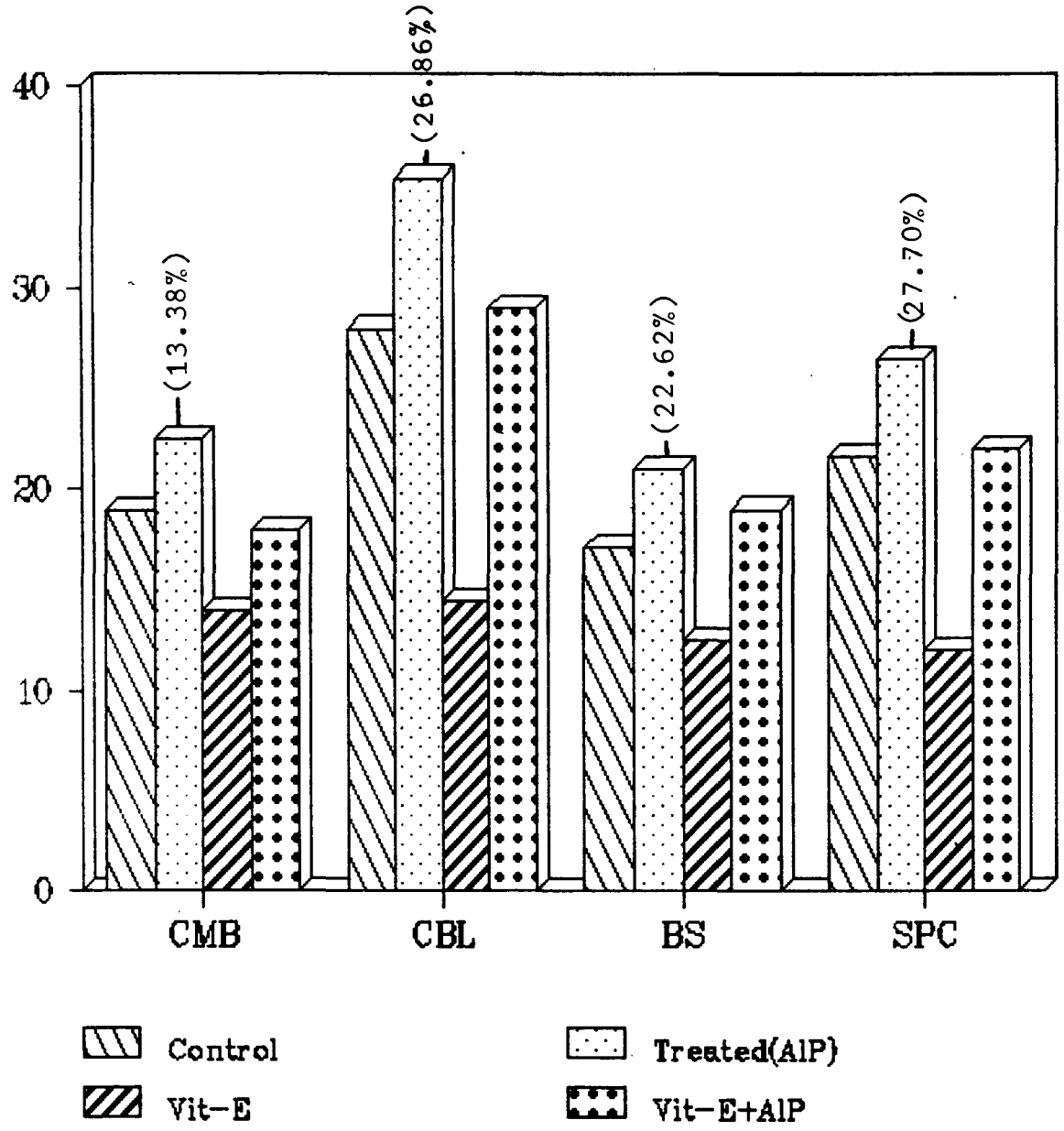


Fig. No.7.1

Table 8

Alteration of T-SH in different regions of the rat CNS after the exposure to Aluminium phosphide (10mg/Kg.b.wt., orally) daily for 7 days.

The levels of total sufhydryl (μ mole/g tissue) (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	10.614	0.95	8.100	0.54	-23.60	<0.01
CBL	11.050	0.59	8.180	0.43	-26.24	<0.001
BS	10.541	0.84	7.925	0.39	-24.81	<0.001
SPC	10.190	0.45	8.040	0.45	-21.86	<0.01

±SE = Standard Error; n = no. of rats.

4.5.2 Glutathione Reduced (GSH):

Effect of AlP: Table-9 shows the levels of free sulfhydryl group. The concentration of GSH in different parts of the brain and spinal cord is decreased after AlP toxicosis. The maximum reduction was noticed in CBL by -20.74% and less in CBM by -15.53%. The increasing rank order was: SPC<CBM<BS<CBL.

Effect of Vitaimn E: Vitamin E administration (150 IU/kg b.wt.) was observed to induce significant of GSH contents in different regions of CNS ($p<0.01$) (Fig.9.1).

4.5.3 Oxidized Glutathione (GSSG):

GSSG levels in different regions of brain and spinal cord following AlP intoxication are given in table 10. The levels of GSSG in different regions of CNS showed values increasing in the following order: CBL> BS> CBM> SPC.

Maximum increment was found in CBL by 24.36% following BS by 23.10%. In cerebrum the rise in the levels of GSSG was 20.40% and in spinal cord 19.31%.

Table 9

Alteration of Free-SH (GSH) in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days.

The levels of GSH (μ moles/g tissue) (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	4.050	0.15	3.421	0.18	-15.53	<0.05
CBL	5.205	0.22	4.125	0.21	-20.74	<0.01
BS	3.921	0.18	3.194	0.20	-18.54	<0.05
SPC	4.150	0.09	3.450	0.17	-16.86	<0.05

±SE = Standard Error; n = no. of rats.

Protective Role of Vitamin-E on the Levels of GSH (150 IU/kg.b.wt., Orally)

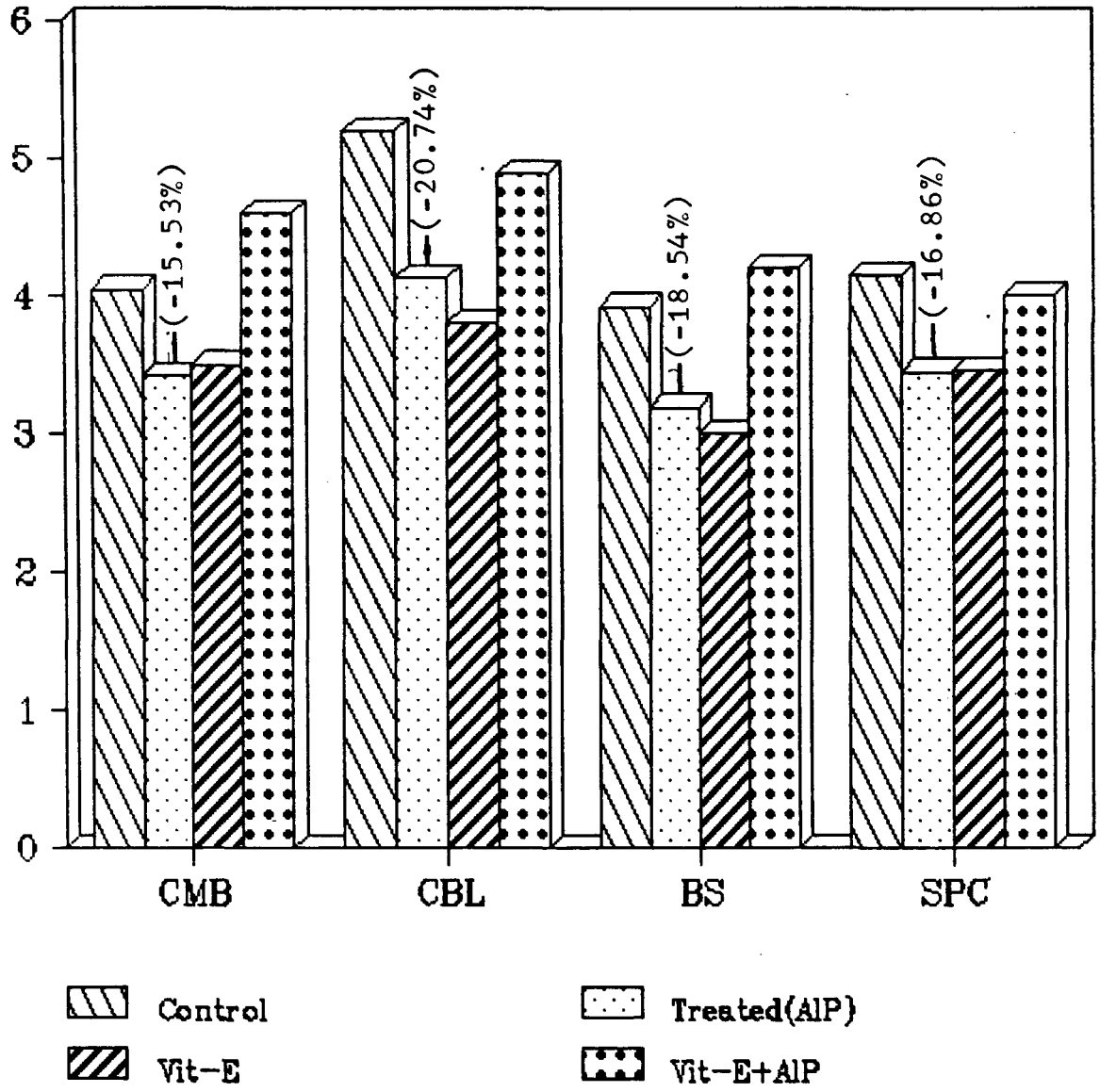


Fig. No. 9.1

Table 10

Alteration of Oxidized Glutathione in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days.

The levels of GSSG (µm/g tissue) (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	2.284	0.06	2.750	0.05	20.40	<0.01
CBL	2.569	0.05	3.195	0.04	24.36	<0.001
BS	2.116	0.07	2.605	0.08	23.10	<0.01
SPC	2.175	0.09	2.595	0.06	19.31	<0.05

±SE = Standard Error; n = no. of rats.

Protective Role of Vitamin-E on the Levels of GSSG (150 IU/kg.b.wt., Orally)

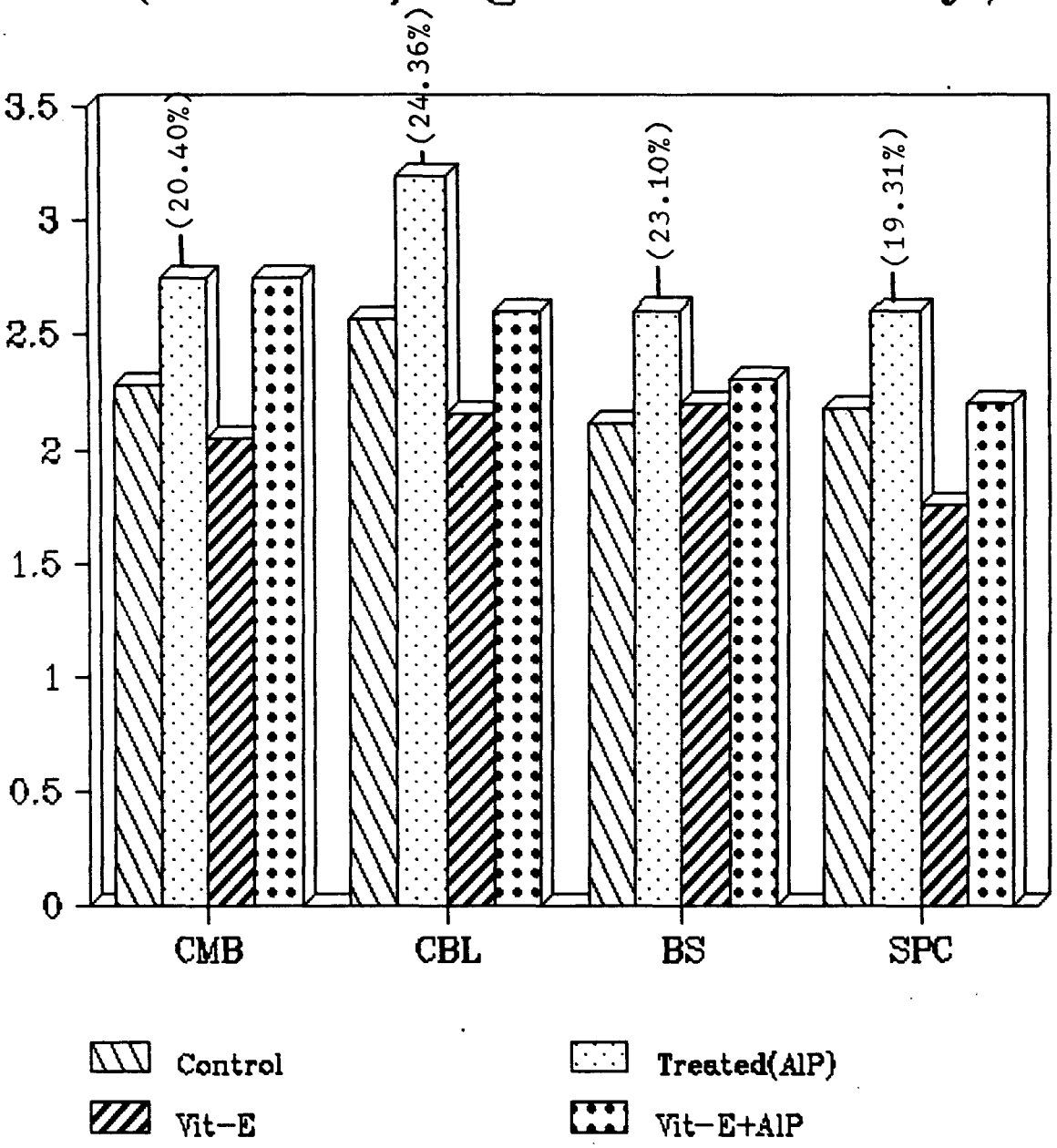


Fig. No.10.1

Effect of Vitaimin E: Fig.10.1showes reduced levels of GSSG after treatment with vitamin E (150 IU/dg. b.wt.) alone ($p<0.001$). When vitamin E was given with AIP solution, a remarkable protection against the elevation of GSSG was observed in various regions of the CNS of tats ($p<0.001$).

4.5.4 GSSG/GSH Ratio:

The ratio of GSSG/GSH are given in table 11. In CBL the ratio is maximum followed by BS,SPC & CBL ($p<0.001$).

4.5.5 Superoxide Dismutase (SOD):

The SOD activity was found to decrease in all the regions of brain and spinal cord followed by AIP treatment (Table-12).

The activity of SOD was maximally depleted in brain stem by (-56.47%) followed by cerebrum(-46.00%), CBL (-42.25%) and SPC (-13.02%).

Effect of Vitamin E: Protective effect of vitamin E against AIP induced inhibition of SOD is given in Fig.12.1 Following the simultaneous treatment of vitamin E (150

Table 11

Alteration of the ratio of GSSG/GSH in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days

The ratio of GSSG/GSH (n=6)				
Regions	**Control**	**Treated**	**%Change**	**pValue**
CBM	0.564	0.804	42.55	<0.01
CBL	0.493	0.774	59.99	<0.001
BS	0.539	0.815	51.20	<0.001
SPC	0.524	0.752	43.546	<0.01

±SE = Standard Error; n = no. of rats.

Table 12

Alteration of SOD activity in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days.

The SOD activity (Units/mg protein) (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	2.249	0.042	1.21	0.036	-46.00	<0.001
CBL	2.020	0.03	51.16	0.038	-42.25	<0.001
BS	2.300	0.029	1.02	0.040	-56.47	<0.001
SPC	2.690	0.025	1.86	0.020	-31.0	<0.01

±SE = Standard Error; n = no. of rats.

Protective Role of Vitamin-E on the Activity of SOD (150 IU/kg.b.wt., Orally)

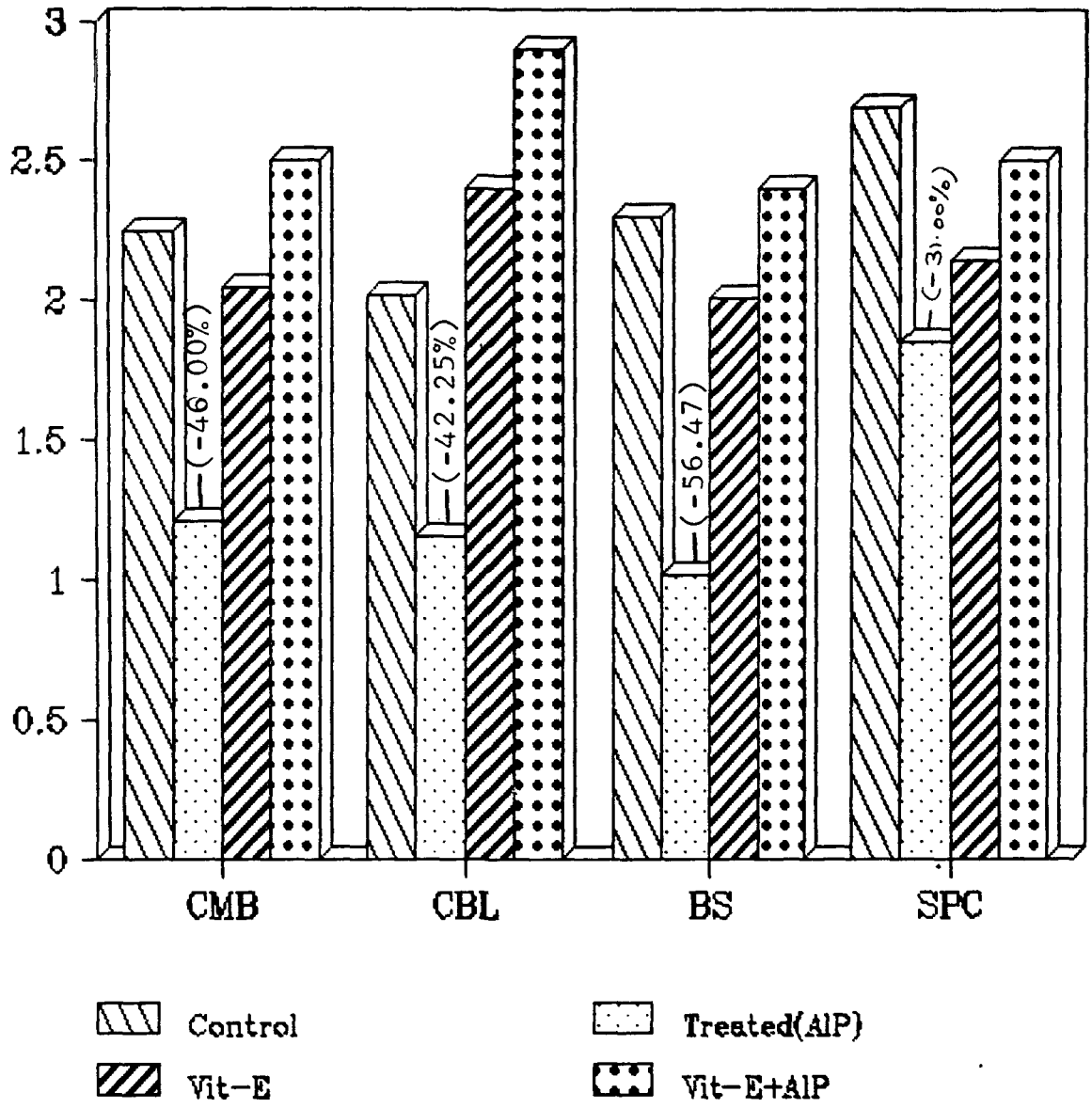


Fig. No.12.1

IU/kg. b. wt., ip) and AIP, no significant change in the SOD activity was observed.

4.5.6 Glutathione Reductase (GR):

The activity of GR in various CNS parts are showed in table 13 after AIP intoxication. The maximum depletion in the activity of GR was foud in CBL and BS (-22.89% and -17.81% respectivley).

Effect of Vitamin E: After treatemnt of vitamin E (150 IU/kg. b. wt. ip.) significant elevation of GR activity ($p<0.001$) was observed in different regions of rat brain and spinal cord (Fig13.1).

4.57 Glutathione Peroxidase (GSHPx):

The GPx activity followed by AIP toxicity in different regions of CNS is given in table 14. The maximum depletion by -21.08% in the activity of GSHPx was noted in BS followed by SPC,CBM and CBL.

Effect of Vitamin E: Protective effect of vitamin E showed significant elevation of above mentioned enzyme in various regions of CNS. But there was no effect of vitamin E against the AIP induced depletion of GSHPx activity (Fig14.1)

Table 13

Alteration of GR activity in different regions of the rat CNS after the exposure to Aluminium Phosphide (10 mg/Kg.b.wt., orally) daily for 7 days.

The GR activity (n mole of NADPH oxidized/min/mg protein) (n=6)						
Regions	Control		Treated			
	Mean	±SE	Mean	±SE	% Change	pValue
CBM	25.61	1.15	20.08	1.22	-21.58	<0.01
CBL	27.29	1.10	21.04	1.51	-22.89	<0.01
BS	23.98	1.20	19.84	1.25	-17.81	NS
SPC	19.52	0.68	15.75	0.70	-19.32	<0.05

±SE = Standard Error; n = no. of rats; NS = not significant.

Protective Role of Vitamin-E on the Activity of GR (150 IU/kg.b.wt., Orally)

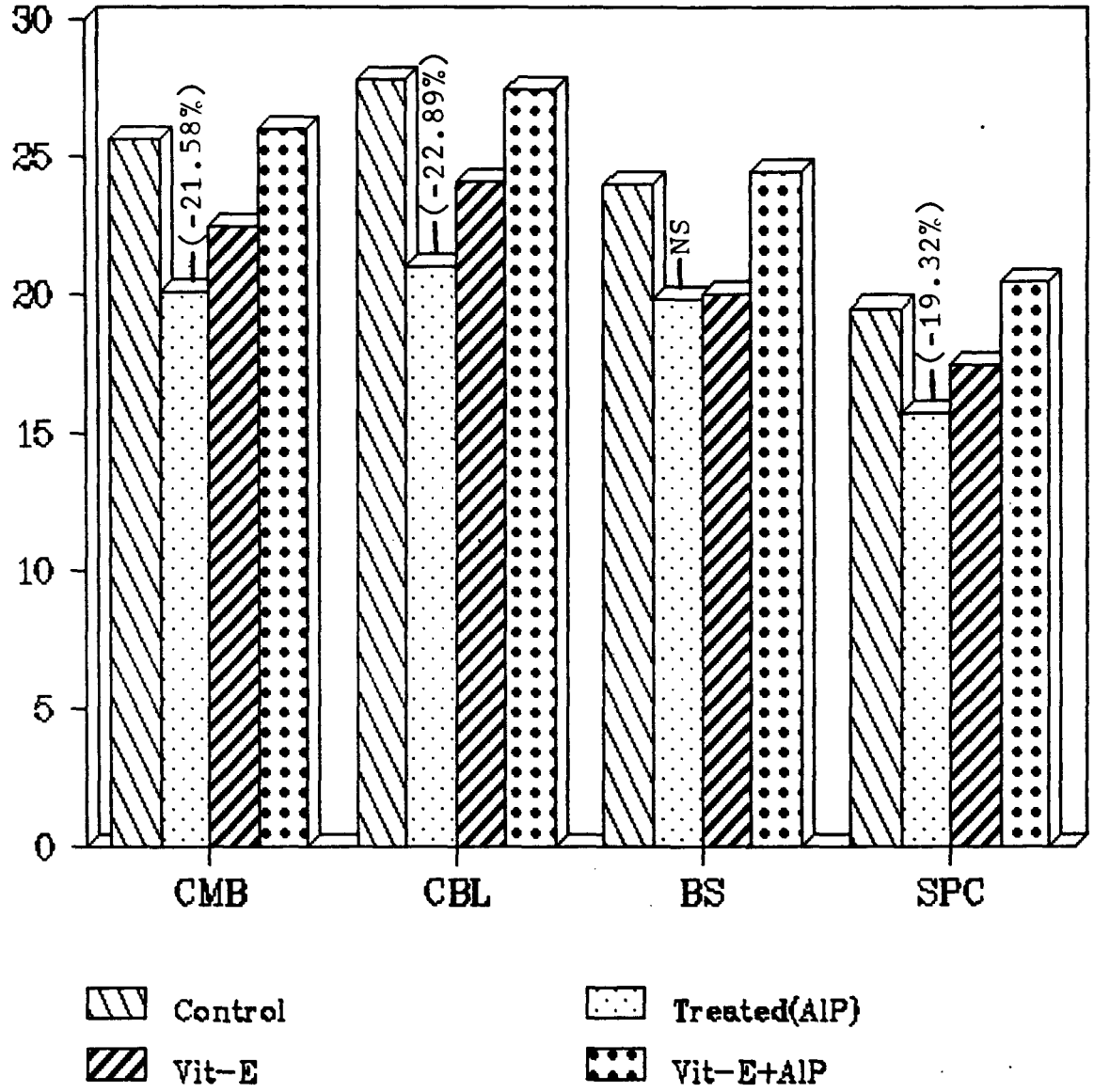


Fig. No. 13.1

Table 14

Alteration of GSHPx activity in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days.

The GSHPx activity (n mole of NADPH oxidized/min/mg protein) (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	47.610	2.12	38.390	2.65	-19.36	<0.05
CBL	81.850	2.90	66.14	02.85	-19.19	<0.05
BS	85.490	2.39	67.465	1.79	-24.08	<0.01
SPC	94.960	3.15	75.380	3.65	-20.62	<0.01

±SE = Standard Error; n = no. of rats.

Protective Role of Vitamin-E on the Activity of GSHPx (150 IU/kg.b.wt., Orally)

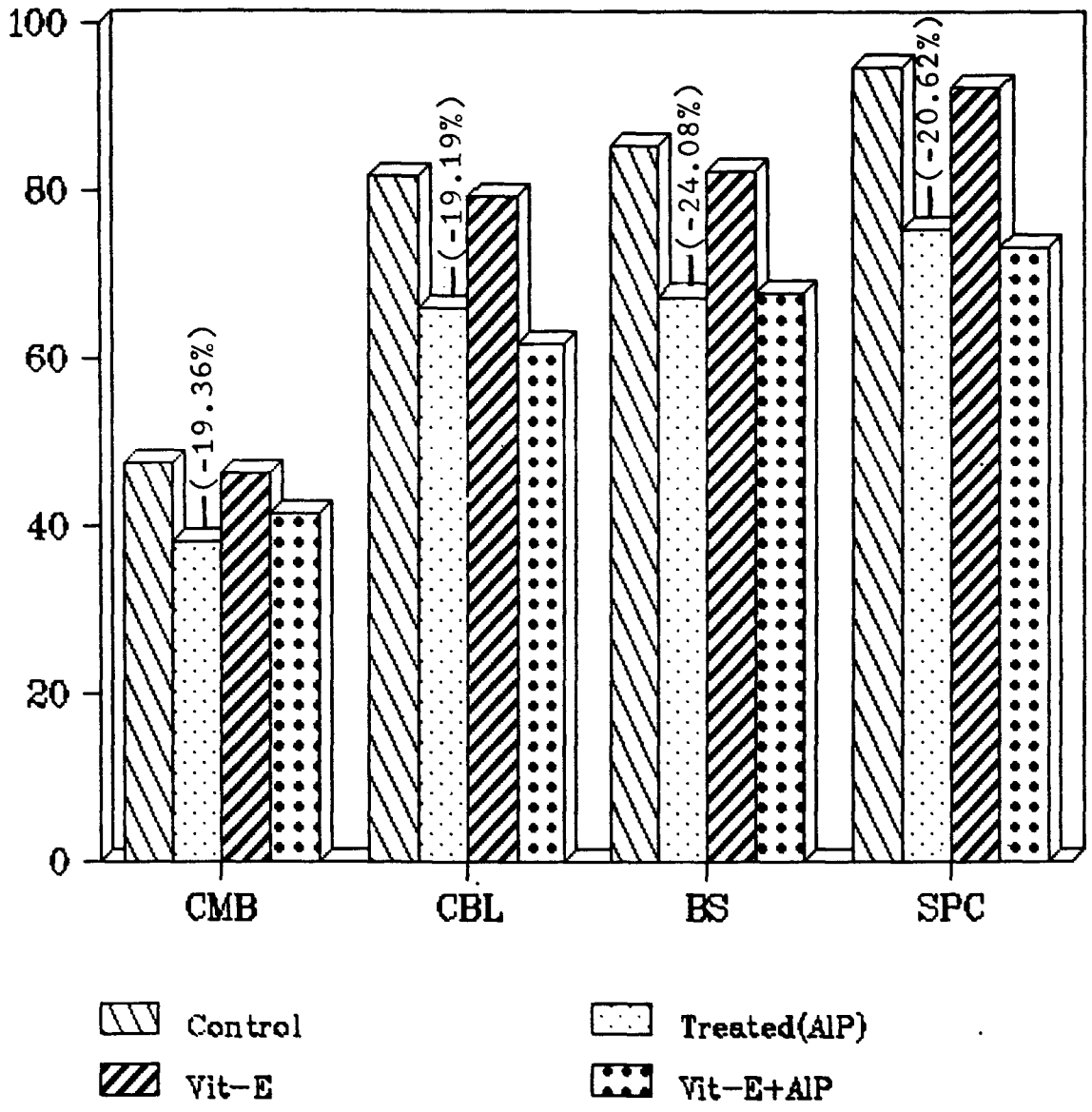


Fig. No. 14.1

4.5.8 Glutathione-S-Transferase (GST):

The significant depletion in the activity of GST was observed in different regions of rat CNS following AIP intoxication (Table 15).

The maximum inhibition was noticed in CBL (-28.96%) followed by CBM (-25.59%) SPC (-24.48%) and BS (-22.56%).

Effect of Vitamin E: Protection by Vitamin E against AIP toxicosis is not significant in different regions of rat brain and spinal cord (Fig.5.1.) in relation to GST activity.

4.5.9 Monoamine Oxidase (MAO):

Table 16 showed enhancement of MAO activity in different regions of rat CNS followed by AIP administration. The activity of MAO was maximally increased in brain stem (22.27%) and minimally in spinal cord (15.92%). In CBL and CBM the MAO activity was almost equal (20.05% and 19.15%).

Table 15

Alteration of GST activity in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days.

Regions	Control		Treated			
	Mean	±SE	Mean	±SE	% Change	pValue
CBM	156.540	3.95	116.47	4.30	-25.59	<0.001
CBL	150.980	4.25	107.25	3.95	-28.96	<0.001
BS	135.10	5.65	104.52	5.12	-22.56	<0.01
SPC	144.78	7.01	108.81	3.65	-24.48	<0.01

±SE = Standard Error; n = no. of rats.

Protective Role of Vitamin-E on the Activity of GST (150 IU/kg.b.wt., Orally)

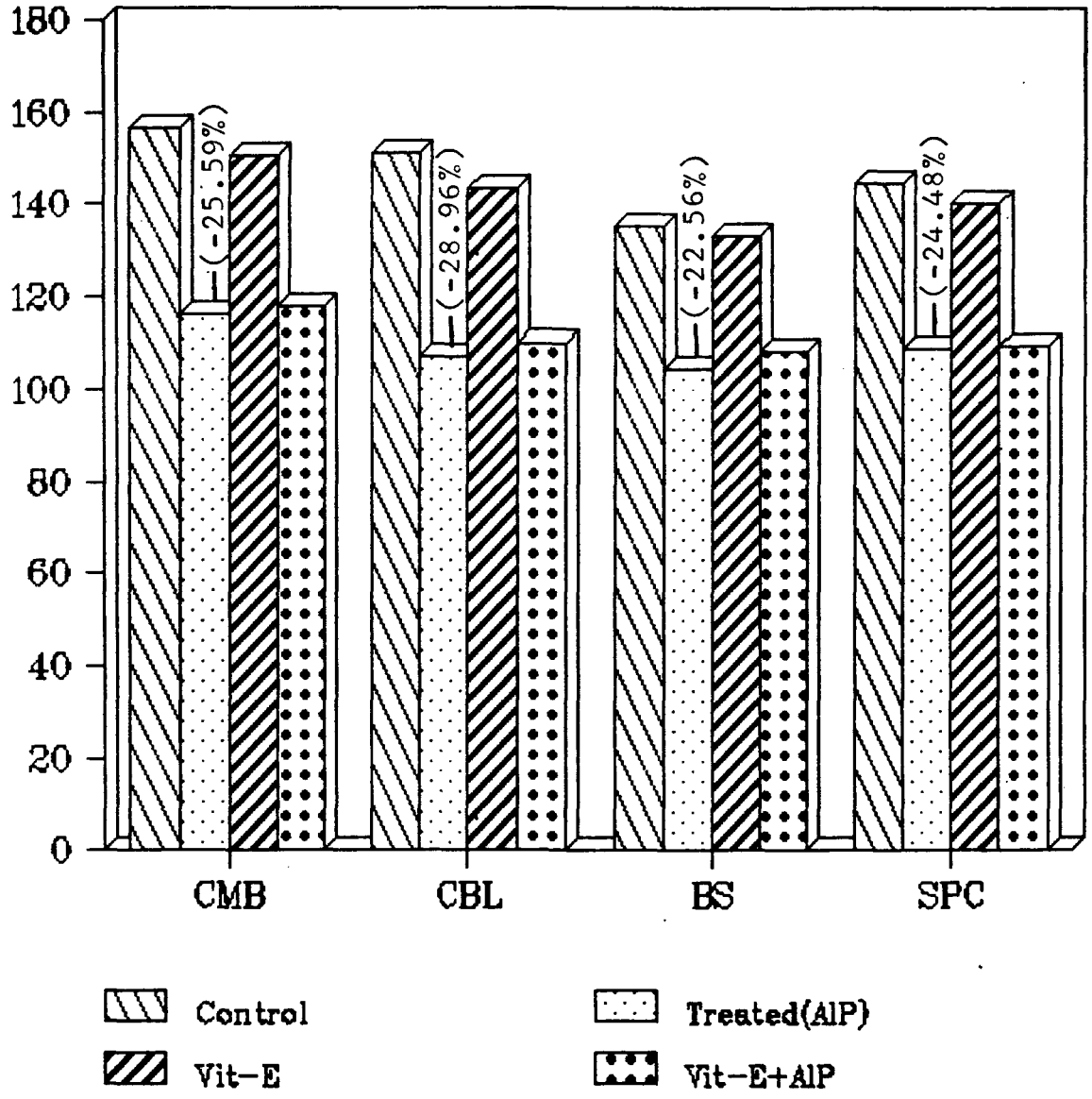


Fig. No. 15.1

Table 16

Alteration of MAO activity in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days.

The MAO activity (n mole of benzaldehyed/min/mg protein) (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	2.182	0.063	2.598	0.034	19.15	<0.05
CBL	2.074	0.045	2.490	0.038	20.05	<0.05
BS	1.805	0.036	2.207	0.040	22.27	<0.01
SPC	1.639	0.049	1.900	0.04	15.92	NS

±SE = Standard Error; n = no. of rats; NS = not significant.

Protective Role of Vitamin-E on the Activity of MAO (150 IU/kg.b.wt., Orally)

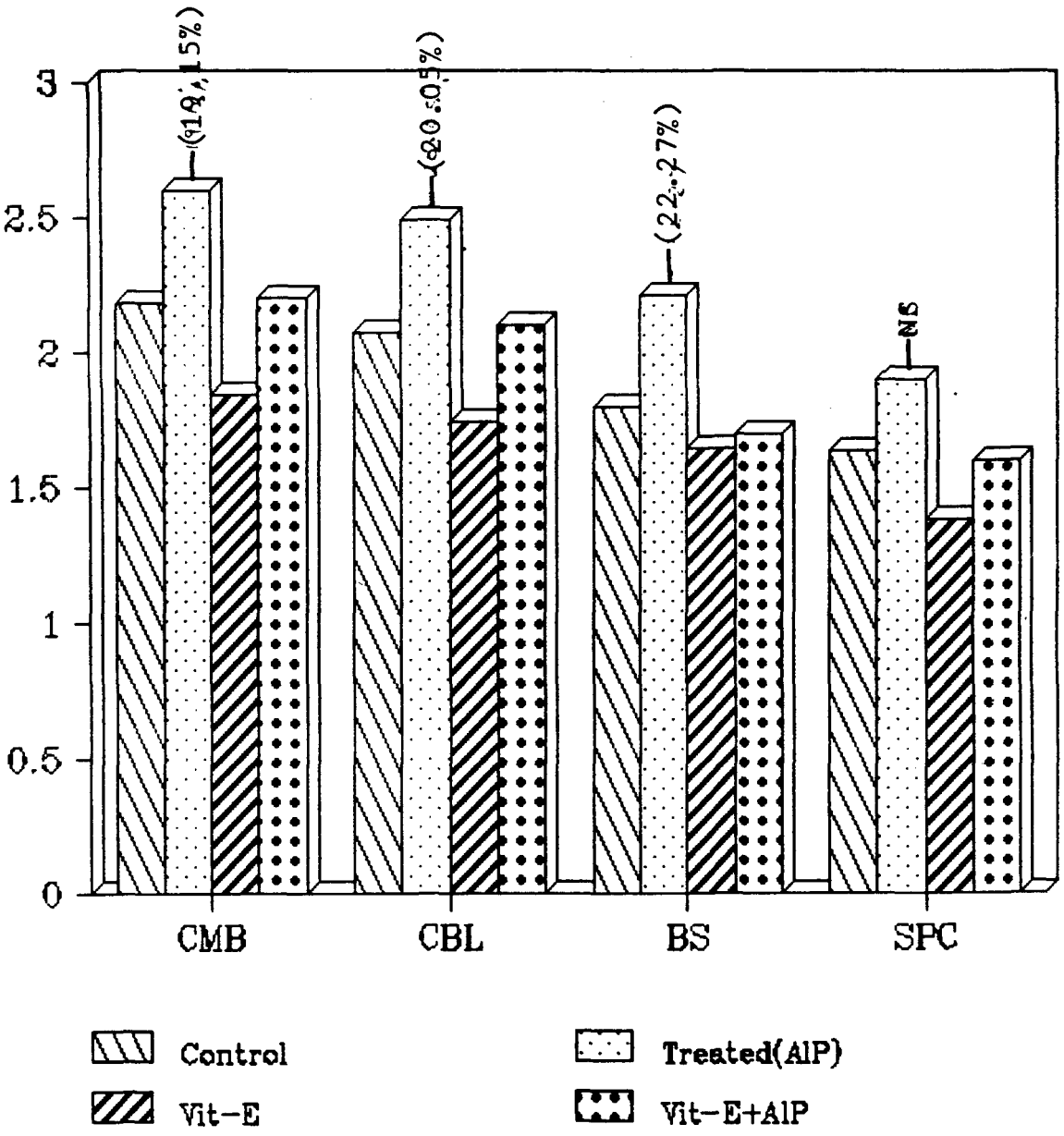


Fig. No. 16.1

Effect of Vitamin E: After treatment of vitamin E (150 IU/kg b.wt., ip). Significant elevation of MAO activity was observed in all the regions of rat CNS (Fig16.1)

4.6 Nucleic Acids :

4.6.1 Deoxyribonucelic Acid (DNA) :

DNA concentration was found to be decreased signigicantly in different regions of the CNS of AIP treated rats (Table- 17). The maximum depletion was observed in CBL (-22.99%) followed by SPC (-22.62%), CBM (-21.78%) and BS (-19.04%).

4.6.2 Ribonucleic Acid (RNA):

The neurochemical alteration in the levels of RNA was observed following AIP toxication. The maximum increase in RNA content was seen in cerebellum (31.43%) followed by spinal cord (27.01%) (Table- 18).

4.6.3 RNA/DNA Ratio:

The RNA/DNA ratio in different parts of the CNS following AIP toxicosis is mentioned in table 19. The maximum value was found in the CBM (1.37 fold)

Table 17

Alteration of levels of DNA in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days.

The levels of DNA (mg/g wt of wet tissue) (n=6)						
Regions	Control		Treated			
	Mean	±SE	Mean	±SE	% Change	pValue
CBM	2.901	0.051	2.382	0.034	-21.78	<0.01
CBL	5.986	0.07	04.370	0.065	-26.99	<0.001
BS	1.841	0.056	1.491	0.060	-19.04	<0.05
SPC	1.790	0.045	1.385	0.030	-22.62	<0.01

±SE = Standard Error; n = no. of rats.

Table 18

Alteration of levels of RNA in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days.

The levels of RNA (mg/g wt. of wet tissue) (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	2.680	0.038	3.266	0.09	18.65	<0.05
CBL	1.985	0.049	2.609	0.08	31.43	<0.001
BS	1.662	0.025	2.029	0.06	22.08	<0.01
SPC	1.440	0.029	1.829	0.08	27.01	<0.001

±SE = Standard Error; n = no. of rats.

Table 19

Alteration of levels of RNA/DNA in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt., orally) daily for 7 days

The ratio of RNA/DNA (n=6)				
Regions	**Control**	**Treated**	**%Change**	**pValue**
CBM	0.924	1.371	48.37	<0.01
CBL	0.331	1.360	50.60	<0.001
BS	0.903	1.360	50.60	<0.01
SPC	0.804	1.320	64.17	<0.001

±SE = Standard Error; n = no. of rats.

followed by BS (1.36 fold), SPC (1.32 fold) and CBL (0.6 fold).

4.7 Protein:

Table 20 showed that the AIP toxicosis led to significant decrease in the total protein contents of CBM (-26.62%), CBL (-23.65%), BS (19.70%) and SPC (-17.52%).

4.8 Acetylcholinesterase (AChE):

AlP toxicosis was found to induce significant inhibition of AChE (Table 21) Depletion of AChE was observed maximum in SPC (-28.75%) followed by BS (-26.23%) and CBL (-22.43%).

Trends of two way **ANOVA** revealed that all the variations, between various regions as well control and treated rats ($p<0.05$) were significant.

Table 20

Aiteration of levels of Protein in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days.

The levels of Protein (mg/g weight of wet tissue) (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	89.900	3.100	65.960	2.800	-26.62	<0.001
CBL	94.620	4.380	72.260	3.090	-23.63	<0.01
BS	70.960	4.680	56.980	2.960	-19.70	<0.05
SPC	69.896	2.906	57.650	2.700	-17.52	NS

±SE = Standard Error; n = no. of rats; NS = not significant.

Table 21

Alteration of the activity of AChE in different regions of the rat CNS after the exposure to Aluminium phosphide (10 mg/Kg.b.wt, orally) daily for 7 days.

AChE activity (µm acetylthiocholine iodide hydrolysed/min/mg protein (n=6)						
Regions	**Control**		**Treated**			
	Mean	**±SE**	**Mean**	**±SE**	**% Change**	**pValue**
CBM	3.215	0.070	2.801	0.040	-12.83	NS
CBL	1.021	0.05	0.792	0.025	-22.43	<0.01
BS	2.985	0.03	2.202	0.080	-26.23	<0.001
SPC	1.986	0.022	1.415	0.030	-28.75	<0.001

±SE = Standard Error; n = no. of rats; NS = not significant.

DISCUSSION

Discussion

5.1 Open Field Behaviour (OFB):

The present study show remarkable changes in the OFB of the AlP treated rats. Significant day to day depletion was noticed in the scores of OFB parameters, i.e. ambulation, preening and rearing.

Ambulation and rearing are known to be horizontal (simple) and vertical (complex) stereotype respectively (Gupta and Holland 1972). Decrement of ambulation (table-1) and rearing (table-3) scores can therefore be correlated with the increment of MAO activity (table-16) as found in my study, as MAO is the main modulator of stereotype behaviour (Kulkarni and Dandiya, 1972). Rearing is also indicative of cortical stimulation (Lat, 1965), while preening response has been described as a behavioral correlate of corticall stimulation (Gupta et al., 1971). Depression of preening score (table-2) can therefore be related to the decrement of cortical

stimulation, i.e. rearing. Our results are further supported by the findings of Naqvi and Hasan (1991b), showing depletion in the OFB parameters alongwith decrease of brain monoamines following intoxication of dimecron, a organophosphate pesticide. The dose response sings of AlP toxicosis, viz dribbling of saliva from mouth, tachyphnoea, restlessness and lethargy are also in agreement with decrease in the OFB parameters.

Remarkable protection against the OFB change was observed following simultaneous treatment with α-Tocopherol (vitamin-E) alongwith AlP, scores of ambulation, preening and rearing, figure 1,2, and 3 respectively showed no significant alteration throughout the seven days of treatment. This protection is well supported by the AlP induced increment of MAO (Fig16.1) with the protection against behavioral changes.

AlP pesticide (fumigant) have been studied regarding their cardiovascular, renal and respiratory effects on the non-target organism as well as dealing of clinical cases, which were admitted in hospitals. No AlP study has been reported on CNS and very little literature

is available, [illegible] on the neurotoxicity of AlP (Qickphos, Celphos).

My present study demonstrates regional heterogeneity not only related to the effects of AlP on various neurochemical and neurobehavioral parameters, but also in the extent of these adverse effects on different parts of the brain and spinal cord. It is noteworthy that the brain is a heterogeneous organ, more correctly; it is collection of many diverse organs rather than a single entity (Hertz, 1969). This heterogeneity is of great importance in the evolution and interpretation of the biochemical and behavioral findings. To understand the neurotoxicity of AlP a number of different parameters were used. The lipid profiles. (Total lipids, cholesterol), lipid peroxidation and LHPO, sulfhydryl group (-SH), antioxidant enzymes (SOD, GSH, GSHPx, GST), MAO, nucleic acid (DNA and RNA) and acetyl cholinestrase activity were evaluate in the different regions of the CNS of albino rats. The protective effect of vitamin E (α-Tocopherol) on some biochemical parameters was also studied.

5.2 Neurobiochemical Changes:

5.2.1 Brain Lipids: Effect of AlP:

The brain lipids in the different regions of the CNS form an important part of neurobiochemical investigations. The vertebrate CNS is richest in cholesterol, sphingolipids, and glycerophospholipids. Lipids play a vital role in both the structure and function of neuronal membrane.

In the present study we found decrement of total lipid, cholesterol, enhancement of lipid peroxidation and LHPO in the different region of brain and spinal cord following the administration of AlP solution (by gavage, daily for 7 days). Todate no literature is availabe on the dose related changes of the CNS following AlP neurotoxicity.

Ordy and Kaack (1975) and Bourre et al, (1990) have described brain lipids which in turn, play a role in modulating the cell structure and function of its biomembranes. Lipids are major constituents of myelin, and also constitute structural elements of the plasma membrane. Myelin metabolism may be affected by a

variety of toxic agents. These disturbances caused effects directly on myelin forming cells, or demyelination may be secondary response to a disturbance of neuronal metabolism. Myelin sheaths and the neuropil account for much of the brain lipids (Robins et al., 1956) Alterations in the lipid metabolism may be due to the changes in the rate of anabolism, catabolism or both, These processes are regulated by the activities of appropriate enzyme systems (Horakova and Steenken, 1994).

5.2.2 Total Lipids:

Total lipids of brain showed AlP dependent depletion in different regions of the rat CNS investigated. Most affected part of the brain is cerebellum where maximum depletion was found. This can be very well explained on the basis of the findings of Majno and Larnovsky (1955), who reported perturbed lipid synthesis in the brain after organophosphate administration. Diminuation of total lipids suggests a possible increase in the degradation of lipids, as evident by the increased rate of lipid peroxidation and lipase activity following organophosphate neurototoxicity

(Islam, et al. 1983; Tayyaba and Hasan 1985, Hasan and Khan 1985; Vadhwa and Hasan 1986; Naqvi, et al. 1988; Milan and Zuzana, 1996; Masayuki, et al. 1996). Ham and Rose (1969) have suggested that changes in the lipase activity may be an important factor contributing to perturbed plasma lipid levels. Furthermore, the decrease in total lipid contents in different regions of CNS of the rat, irrespective of their regional variations, can be explained on the basis of the observation of Caley and Jenson, (1973), who detected inhibition of lipase activety following OP administration.

5.2.3 Cholesterol:

The high concentration of cholesterol seems to be characteristic of CNS. It occurs in white matter in amounts exceeding those in gray matter (Brante, 1949). It is the characteristic lipid of myelin sheath. About 70% cholesterol of the brain is present in the myelin (Dobbing, 1963). The constant amount of cholesterol in the brain suggests that the sterol is metabolically stable and is thus removed from the normal dynamic exchange process common to almost all other body constituents

(Waelsch et al., 1940). Radioactive isotope studies performed by Dawson (1954), show that both lipid fatty acids and phospholipids, respectively are metabolically more active than cholesterol in rat brain.

Dose dependent inhibition of cholesterol level is observed in all the different regions of the rat CNS following chemical stress. These observations were supported by the earlier studies of Prasada and Ramana (1984); and Gupta, A. (1992). The decrement in cholesterol level can be explained on the basis of either its increased synthesis or decreased. This is well in agreement with the AlP induced decrease in the content of cholesterol in various regions of the CNS.

5.2.4 Lipid Peroxidation:

Lipid peroxidation, being the basic mechanism of destruction of usaturated fatty acid chains in biological membranes, is maintained in the organism on a required level by a contribution of several controlling factors. The peroxidation of endogeneous phospholipids in biological membranes has long been thought to be the basis for a variety of toxicological phenomenon. For example, Lipid

peroxidation has been implicated in the damage to cells that results from their exposure to air pollutions, chlorinated hydrocarbons, oxident haemolytic agents, ethanols and even organophosphates. Lipid peroxidation appears to be a phenomenon that reflects free radical events associated with biological membranes, which contain most of the polyunsaturated fatty acid-containing lipids in animal tissues (Goldstein, et al. 1993). The brain homogenate has apparently the necessary unsaturated fatty acids and the catalysts for peroxidation in the architecture of the cell itself which are readily available for reaction with molecular oxygen to undergo lipid peroxidation. Peroxidation involves the direct reaction of oxygen and lipid to form free redical intermediates and to produce semistable peroxides. Kartha and Krishnamurthi (1978) reported that among the different tissues from normal rat the brain showed considerably higher peroxidation.

In present study we found that toxicosis with AlP caused significant enhancement of lipid peroxidation in different regions of brain and spinal cord. This enhancement can be supported by the findings of Hasan

and Ali (1981) Masayuki, et al. (1996) and Stefek, et al. (1992) disclosing increment of the rate of lipid peroxidation following administration of OP dichlorovos (Naqvi et al., 1988). The MDA level was increased in blood serum (Chugh et al., 1992). LPO rate was also increased with the treatement of Quinalphos (Vijaya Kumar T.S., 1994). Elevation of lipid peroxidation can also be correlated with depletion of T-SH groups content in the different regions of the brain and spinal cord following dimecron toxicity (Naqvi and Hasan, 1991), because it has been suggested that decrement of sulfhydryl groups may lead to deficient degradation of lipid peroxides in the tissue (Tappel, 1970).

5.2.5 Effect of Vitamin E on the Rate of Lipid Peroxidation Following the Administration of AIP:

Although Vitamin E is well established as a fat soluble scavenger of lipid peroxyl radicals, protecting biological membranes from peroxidation (Diplock, 1985), its storage in association with body lipids and its possible function as a biological antioxident suggest that a close relationship exists between vitamin E and various

phases of lipid metabolism. The antivitamin E activity of organophosphates has been questioned (Green and Bynyan, 1969; and Goyer et al., 1970). Some have proposed that tri-ortho cresyl phosphate (TOCP) merely reduces the absorption of vitamin E from intestinal tract, thereby accounting for the lowered blood level of vitamin E characteristic of TOCP poisoning (Myers and Mulder, 1953). Findings of Shull and Cheeke (1973) indicate that the administration of TOCP may influence the utilization of vitamin E, in growing rats, supplementation with vitamin E prevented the growth cessation caused by feeding TOCP. Similarly, in the present study, rats that had ceased growing following AIP administration responded with renewed growth and significant weight gain when vitamin E was administered.

The role of vitamin E as a biological antioxidant and its interrelatonships with some phases of lipid metabolism, point to its possible involvement in certain enzyme systems in the body. It has been suggested that the clinical manifestations of vitamin E deficiency result from the action of lipid peroxides on sulfhydryl groups

of various enzymes, thus inhibiting their activity.

A significant body of evidence indicates that the primary if not the sole, function of vitamin E in metabolism is that of an "in vivo" lipid antioxidant. Probably the most direct evidence to substantiate this theory is that lipidperoxides have been found in the tissues of vitamin E deficient animals (Dam and Granados, 1945). It is assumed that vitamin E acts as an "in vivo" lipid antioxidant, protecting unsaturated fatty acids in tissue lipid against peroxidation. The chemical process of peroxy free-readical reaction with an antioxidant (vitamin E) to terminate the reaction chain is defined as follows:

$$\underset{\text{peroxy free radical}}{LOO^{\bullet}} + \text{Vitamin E} \longrightarrow LOOH + \underset{\text{Oxidized}}{\text{Vitamin E}}$$

Bieri and Anderson (1960) suggested that each tissue has an "antioxigenic potential" which was determined by a balance between factors promoting peroxidation and by those exerting an antioxidant action.

According to Combs et al. (1975) and Meydani et al. (1993) at least two systems are important for uncontrolled lipid peroxidation. These systems rely on selenium and vitamin E, respectively and form the basis for the hypothesis concerning the antioxidant functions of these nutrients (Combs et al., 1975). It appears likely that a similar mechanism operates in the AIP induced increase rate of lipid peroxidation was observed in the present study and its protection by vitamin E.

The peroxidative changes triggered by free radicals in brain fatty acids and phospholipids may be of importance in the development of brain cell damage. Free radicals are constantly being formed in various reactions essential for aerobic life. The best studied effect of free-readical attack is that causing lipid peroxidation i.e. oxidation of methylene bridges of unsaturated fatty acids, resulting in the formation of lipidperoxides and hydroperoxides, finally leading to fragmentation of lipids. Vitamin E (α-Tocopherol) is known to stabilize plasma membranes (Porta et al., 1968; Gey et al. 1991) as well as lysosomal (Brown and Pollocle, 1972) and also mitochondrial membranes (Frigg

and Rohr, 1976). In the present investigations the administration of α-tocopherol alone caused significant reduction of the rate of lipid peroxidation, and in combination with AIP, prevented the increase of lipid peroxidation.

5.2.6 Sulfhydryl Groups (-SH):

To date, no report is available on the estimation of sulfhydryl groups in different regions of rat brain following AIP administration. Sulfhydryl groups are known to act as active enzymatic sites (Hoch and Vallee, 1959). Sulfhydryl enzymes have been observed to be the most susceptible to lipid peroxidation induced inactivation (Chio and Tappel, 1969). Glutathione (GSH) has been reported to play a protective role against free radical-mediated peroxidative damage (Stokinger and Coffin, 1968; Little and O'Briene, 1968; Delucia et al., 1972; Chow and Tappel, 1972). GSH which is a potent nucleophile is involved in conjugation reactions which are important for detoxication against electrophilic toxicants (Chasseaud, 1980). This conjugation reaction between -SH group of glutathione and the toxic

compound has been reported to be catalyzed by an enzyme glutathione-s-transferase (GST) (Habing et al., 1974; Al-Turk et al., 1987).

The concentration of glutathione of tissues has been reported to correlate positively with the longevity of the organisms (Long et al.,1989; Sohal et al., 1990; Masayuki, et al. 1996; and Cutler, 1991). GSH protects cells from the toxic effects of reactive oxygen species or peroxidative damage (Little and O' Brien, 1968; Chow and Tappel, 1972) and participate in the preservation of thiol disulfide status of protein (Sohal et al., 1990 and Meister, 1988).

The present study indicates significant depletion in the concentration of T-SH (Table-8) in all the CNS regions following AIP intoxication. Sulfhydryl group is essential for the function of a number of enzymes and proteins.

Free sulfhydryl group (GSH) inhibition (Table-9) were also observed in different parts of brain and spinal cord with toxicosis of AIP. This depletion is correlated with the study of Hazeltan and Lang, (1980) on mammalian tissues.

In 1990, by Sohal et al., demonstrated that the GSH levels has been decreased in developing brain of rats. GSH may be the key factor in lowering reducing potential which occurs in senescent tissue. In support of this argument is the evidence that cellular GSH concentration may have a profound regulatory effect on the activity of pentose phosphate cycle enzymes (Hosoda and Nakamura, 1970). These results indicate a decrease in the 'de novo' synthesis of GSH from amino acids and a concomitant increase in GSSG (Thompson, 1992). Overall the depletion of GSH pool results in dysfunction of the oxidative defence mechanisms. Parallel to decrease in the GSH levels, we observed an increase in the level of lipid peroxides, lipid hydroperoxides in different regions of CNS.

The concentration of oxidized glutathione (GSSG) was elevated significantly in different regions of brain and spinal cord followed by AIP toxicity (Table-10). These results are in agreement with the observation of Oeriu and Tigheciu (1964) and Barlo-Walden et al. (1995), who reported that the elevation of GSSG due to autoxidation of age factor.

5.2.7 Superoxide Dismutase (SOD; Superoxide:Superoxide oxidoreductase, E.C.I.15.1.1) :

It is the first enzyme of the scavenger enzyme series to ameliorate the damage caused in the cells by free radicals (Slater, 1984).

Present study results showed significant changes in enzyme activity, with AIP intoxication. SOD activity was found to be depleted in differnet regions of brain and spinal cord of the rat (Table-12). The results are in accordance with the report of Danh et al. (1983) & Mizuno and Ohta (1986). Superoxide dismutase activity is universally present in respiring cells. The SOD activity is said to be a natural defence mechanism against oxidase damage to the tissue (Fridorich, 1975).

Recently from our laboratory formation of superoxide radicals after chemical stress, a realtively higher decrease in SOD activity was detected (Naqvi and Hasan, 1992).

To date, no report is available on the effect of SOD activity after toxicosis of AIP. However, AIP has been

reported to enhance the lipid peroxidation and degredation of brain lipids. SOD is known to protect against peroxidative damage and deplete -SH groups (Naqvi and Hasan 1991a). This is also in concordance with the diminution of SOD activity in blood serum (Chugh et al., 1992).

Vitamin E (α-Tocopherol), however, revealed remarkable protective effect againt inhibition of SOD activity (Fig12.1). Combined administration of vitamin E and AIP showed significant protection of SOD activity. On the other hand the activity of SOD increased by vitamin E administration alone. Interestingly, in our laboratory protection of lipid peroxidation with vitamin E against metasystox intoxication in different regions of CNS. (Tayyaba and Hasan, 1985), which also supports our findings.

5.2.8 Glutathione Reductase (GR ; NADPH: Oxidized Glutathione Oxidoreductase, E.C.1.6.4.2) :

GR catalizes the production of GSH from GSSG, which accumulates in the cells after the catalytic reduction of oxidative metabolites. Reducing potencial of the cells is maintained by glutathione reductase

(Mantero et al., 1988). Present study results reveals that the GR activity was highest in the rat cerebellum (Table - 13) following AlP toxicosis. These observations are in line with stohs et al., (1984), who reported higher GR activity and GSH content in erythrocytes from young aged humans followed by a considerable decline leading to senescence. This declin to the increased lipid peroxidation (Kotah, et al., 1989). GR activity increased in brain of rat and mice (Hothersall et al., 1981; Kanh, et., 1983 and Tayarami, et al., 1989), but Kellogg and Fridorich (1976), Mizuno and Ohta (1986) and Cand and Verdetti (1989) observed no change in the activity of GR. GR activity can be easily correlated with the decrease in GSH, increase in GSSG levels and GSSG/ GSH ratio. The available data strongly suggest that free radicals are produced during chemical stress by AIP intoxication.

Activity of GR is elevated after the treatment of vitamin CNS of rat. GSH level was also increase with the vitamin E treatment. Hence, the observed α-Tocopherol induced increase in GR activity may be due to an elevation of GSH levels and depletion of lipid peroxidation.

5.2.9 Glutathione-S-Transferase (GST; EC.2.5.1.1C):

GST of aerobic organisms is being hypothesized to have a pivotal role in the detoxification of oxyradicals and their products (Mannerrick and Danielson, 1988). It represents an important class of xenobiotic metabolising enzymes (Mukhtar et al., 1981). There are various isoenzymes of GST and each form may have specific substrate (Lai and Tu, 1986). According to Dixit et al., (1981). GST has been shown to be present in the mammalian and avian brain, besides its wide distribution in the body tissues. Brain GST has been reported to play an important role in the detoxification of potential toxicants through their conjugation and biotransformation (Boyland and Chasseaud, 1969; and Kubota et al., 1985).

In brain the activity of GST was observed to be much higher than other antioxygenic enzymes and hence it may have a prominent role in scavenging oxyradicals and their products. In the present investigation the results showed AlP induced reduction of GST in various regions of rat CNS. Bolyand and Chasseaud (1969), who reported that greater accumulation of the toxic

compound may inhibit the activty of GST.

The present study results also indicate that GST activity increases after the administration of vitamin E. Recently, from our laboratory it was reported that after chemical stress, GST was decreased while the citiolone treatment enhance the GST activity (Naqvi and Hasan, 1991a).

5.2.10 Glutathione Peroxidase (GSHP; NADPH: Oxidized; Glutathione oxidoreductase, E.C.1.11.1.91):

Oxidative damage in biological tissues of aerobic cells may be caused due to the production of peroxides by activated oxygen GSHPx, a selenium dependent enzyme, metabolizes these hydroperoxides and products of cell membrane from peroxidative damage (Mc Cay et al., 1981; Glohe, 1982). The activity of GSHPx is highest in brain areas having catecholaminergic neurons. Cohen (1983) reported that the defence against production of H_2O_2 by monoamine oxidase. Recently, another form of GSHPx, phospholipid hydroperoxide glutathione peroxidase (PHGPx) was reported, which is also selenium dependent enzyme, detoxify the

membrane bonded phospholipid hydroperoxides (Zhang, et al., 1989; Thomas, et al., 1990).

The results of present study indicated that the activity of GSHPx was depleted with AlP toxicosis (table- 14). Regional distribution of the GSHPx activity is also in concordance with earlier reports (Brannan, et al., 1980; Ansari et al., 1989).The inhibition of GSHPx activity due to the passage of time and restraint may predispose brain tissues have a high rate of oxygen consumption, peroxides are formed by the amino oxidase enzymes (Kapller-Adler, 1974). GSHPx activity is also correlated with the lipid peroxidation products.

Due to antioxidant effect of vitamin E the GSHPx activity was elevated in different regions of rat brain and spinal cord. (Fig14.1) while the levels of lipid peroxide and hydroperoxides were decreased.

5.2.11 Monoamine Oxidase (MAO; E.C. 1.4.3.4; Outer membrane enzyme of mitochondria):

Monoamine oxidase plays an important role in the metabolism of biogenic amines and on the behaviour (Sgeller berger and Walaszee, 1972 and Chase and

Murphy 1973). It is involved in the enzymatic deamination and subsequent degradation of amines and results in the formation of H_2O_2 (Cooper, et al., 1978). The concept of two functionally distinct forms of monoamine oxidase were gained wide acceptance in last two decades (Houslay et al., 1976; Holzbauer and Youdin, 1977; Tipton and Della corte, 1979). Type "A" of MAO deaminates neuro transmitter amines of catecholaminergic systems (5-HT and NA), whereas type "B" oxidizes benzylamine and B-phenylethylamine. Substrates such as tyramine and tryptamine are determined by both forms of MAO (Houlay et al., 1976). In the present study no attempt was made to differentiate the activity of two isoenzymes, MAO "A" and MAO "B". Benzylamine hydrochloride was used as a substrate in this study. MAO activity reported, increasing trend in all brain regions and spinal cord with the intoxication of AlP administration. The results of the present study are in accordance with the earlier reports. The increase in MAO activity was observed despite the decrease in the level of GSH and GR increase in lipid peroxidation products.

In 1991, Naqvi and Hasan reported regional alteration in the monoamine oxidase activity following the administration of dimecron. They showed that MAO activity was increased after chemical stress.

A remarkable vitamin E-induced protection against the MAO activity elevation was noticed in all the regions of brain and spinal cord (Fig16.1) The activity of MAO was reported to be near the central values in the CNS regions of rats subjected to simultaneous treatment of vitamin E and AIP. This protection may be the result of detoxication against metasystox neurotoxicity by vitamin E; a potent antioxidant which has been reported to protect against adverse effects of metasystox on brain and spinal cord (Tayyaba and Hasan, 1985).

5.2.12 Nucleic Acids

The AIP intoxication was reported to induce significant perturbations in the levels of nucleic acids viz.-deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in various regions of the brain and spinal cord.

5.2.12.1 Deoxyribonucleic Acid (DNA):

Significant depletion was observed in the concentration of DNA in various regions of CNS after toxicosis with AIP administration (Table-17). DNA seems particularly plausible as a critical target in developing because of the central role of DNA in information transfer between generations of somatic cells. It was shown that different regions of the brain have different DNA concentrations., with the maximum in cerebellum. The findings are in agreement with the observations of May and Grenell (1959).

The found out DNA contents in various CNS regions, thereafter, might be due to AIP induced perturbation in the metabolism of DNA, in addition to the degenerative changes.

5.2.12.2 Ribonucleic Acid (RNA):

In contrast, however, the response of RNA towards AIP poisoning was entirly different from that of DNA. The RNA levels showed a significant increment in all the CNS regions of rat (Table-18).

This increases of RNA concentration may be explained on the basis of the suggestion of Heath (1961), that demyelination, an important future of oganophosphate poisoning, results in the increased production of RNA. Interestingly, it has been demonstrated that the synthesis of RNA increases due to damage of neurons (McIlwain and Bachelard, 1971).

Our results are in agreement with previous reports from our laboratory showed the elevation of RNA in various regions of CNS after chemical stress (Hasan, et al., 1979b; Tayyaba, et al., 1981 ; Naqvi and Hasan, 1992). The elevation of RNA levels is generally associated with the improvement in protein synthesis or tissue function (Prosser, 1969) as well as diminished synthesis of RNA.

5.2.13 Protein:

The total protein levels were significantly depleted in all the different regions of brain and spinal cord with AIP intoxicated rats (Table-20). The present study results were in agreement on the basis of the suggestion of Hyder and Lange (1972), that increased neuronal

activity on inhibition of protein synthesis. According to Ahmad, et al., (1978) the decrement of protein was due to increased proteolytic activity necessiated by greater energy demands under toxic stress.

Moreover, reduction of protein can be correlated well with DNA loss. Bergen, et al., (1974) have also correlated elevated RNA levels with increased utilization of protein, which also supports the AlP-induced depletion of protein. Free amino acids in the brain are known to be involved in a number of metabolic process. Hence the increased demand for amino acids possibly may induce the break-down of proteins, as suggested by Neame (1968).

Previous reports on protein depletion from our laboratory (Tayyaba, et al., 1981), reported diminition of proteins in CNS of rats intoxicated with metasystox. Similar results were obtained in the regions of fish brain and spinal cord, following dichlorvos (Vadhava, 1989). Methyl parathion has also been found to decrease the brain proteins (Khan, 1989). All the

findings correlate with the present study results.

5.2.14 Acetylcholinesterase (AChE; Acetylcholinehydrolase, E.C.3.1.1.7):

AChE is regarded as a tool for quantification of OP-induced effects (Voss, 1968). Inhibition of ChE's caused by OP's poisoning has been noticed in detail earlier but no reports have been available toward AIP poisoning and also mode of action of AIP. The mode of action of OP's in vertebrates is generally regarded as disruption of nerve impulse in the CNS and PNS due to the inhibition of ChE and consequent accumulation of ACh at synapses (Coppage and Matthews, 1975). The resulting disturbances in electrophysiology cause loss of muscular coordination, induction of convulsions and ultimately death. The present study results reveal the inhibitory effect of AIP in different regions of CNS (Table-21).

Previous reports on AChE indicated the inhibitory action of parathion in rat brain (Du Bois, et al., 1949; Emsley, et al., 1976; and Fiscus and Van Meter, 1977). The decrease in the levels of brain

monoamines by Parathion treatment (Naqvi and Hasan 1991b), who also endorses the associated AChE reduction (Naqvi and Hasan, 1991c). Brzezinski (1978) and Brzezinski and Paruszewska (1980) have also reported decrease in both the brain neurotransmitters and increase in the content of acetylcholin (ACh), i.e. it also consistent with the depletion of AChE. Here also reported inhibitory effect of paraoxon, a metabolite of parathion, was found to achieve quick inhibition of the activity of ChE in the brain (Holmstedt, 1959 and Matin, 1974). This is also consistent with our findings in the animals treated with AIP solution.

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